ENDOCRINOLOGY

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VOLUME 26

January–June, 1940

Some day there might be a monument to the Standard White Rat. Millions of human children whom he has saved from suffering and death could dance around it crowned with flowers. Great is the moral grandeur of sacrifice, even when it is unconscious.
Garrett P. Serviss, *Everyday Science*, 1925

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CONTENTS OF VOLUME 26

NO. 1, JANUARY, 1940

Acromegaly and Diabetes Mellitus. *Chester Coggeshall and Howard F. Root* 1
A Comparative Study of the Metabolic Effects of Testosterone Propionate in Normal Men and Women and in Eunuchoidism. *Allan T. Kenyon, Kathryn Knowlton, Irene Sandiford, F. C. Koch and Getrude Lotwin* 26
Observations on the Continued Use of Male Sex Hormone over Long Periods of Time. *Joseph Eidelsberg and Elliott A. Ornstein* 46
The Tolerance of Male and Female Mice Respectively to Estrogens and Androgens. *Charles D. Kochakian* 54
The Composition of Bones of Mice Receiving Estrogens and Androgens. *John H. Wentworth, Paul K. Smith and William U. Gardner* 61
The Mechanism of Estrin Therapy in the Relief of Dysmenorrhea. *Somers H. Sturgis and Fuller Albright* 68
The Relation of Hyperemia to the Action of Estrin. *Oscar Hechter, Maurice Lev and Samuel Soskin* 73
Experimental Studies on the Menstrual Cycle of the Baboon (*Papio Porcarius*). VI. The Effect of Progesterone upon the First Part of the Cycle in Normal Female Baboons. *Joseph Gillman* .. 80
The Potency of Certain Commercial Hormone Preparations. Second Study. *Fred E. D'Amour* .. 88
The Biologic Potency of International Standard Chorionic Gonadotropin. *Fred E. D'Amour and Marie C. D'Amour* 93
The Influence of Various Physiological Substances on the Glycogenolysis of Surviving Rat Liver. The Influence of Cortical Hormone Added in Vitro. *H. P. G. Seckel* 97
The Metabolism of Nitrogen, Sulfur and Glutathione in Thymectomized Albino Rats. *Marta Sandberg, David Perla and Olive M. Holly* 102
The Relationship Between the Thymus and the Sexual Organs. *Hugo Chiodi* 107
The Output of Protein Metabolism Hormone of the Pituitary Anterior Lobe. *Karl E. Paschkis and Annie Schwoner* 117
The Effect of Estrin on the Anterior Pituitary of Male Rats after Pituitary Stalk Section. *Unto U. Uotila* 123
The Regulation of Thyrotropic Function by Thyroxin after Pituitary Stalk Section. *Unto U. Uotila* 129
The Effect of Thyrotropic Hormone upon Pregnant Rabbits. *Beatrice Whiteside* 136
Autonomic Control of Thyroid Secretion. *H. B. Friedgood and W. B. Cannon* 142
A Method of Assaying Pituitary Water Retention Principle. *Eldon M. Boyd and Edward G. Mack* 153
The Effect of the Crustacean Eye-stalk Hormone upon Water Metabolism and Melanophore Expansion in the Frog. *Stephen W. Gray and Walter Ford* 160
Clinical and Laboratory Notes
The Treatment of Pituitary Dwarfism with Growth Hormone. *Joseph M. Looney* 163
The Response of the Ovaries to Gonadotropic Extracts in Splenectomized Rats. *Frederick E. Emery, Thaddeus J. Bugelski and Edward L. Schwabe* 167
Correlation of Pituitary and Spleen in Experimental and Control Mice. *J. M. Twort and R. Lyth* 170
Association Notice 172
Current Endocrine Literature 175

NO. 2, FEBRUARY, 1940

Studies on the Mechanism of Diabetic Disturbance Using the "Nitrogen Minimum Excretion" as a Measure of Glyconeogenesis. *S. J. Thannhauser* 189
Oral Use of Pregneninonol in Functional Meno-Metrorrhagia. *E. C. Hamblen, N. B. Powell, W. Kenneth Cuyler and C. J. Pattee* 201
Comparison of Different Preparations of Amorphous and Zinc Crystalline Insulin. *Frederick M. Allen* 208

Three Cases of Masked Hypothyroidism Having Abdominal Symptoms. *Anthony Bassler* 218
The Medical Treatment of Hyperthyroidism. *Walter Redisch and William H. Perloff* 221
A Case of Acromegaly Studied During the Course of the Disease and at Autopsy. *Gerald Flaum and Elaine P. Ralli* 229
The Inhibition of Lactation Post-Partum with Testosterone Propionate. *Jacob S. Beilly and Samuel Solomon* 236
Muscle Strength in Myotonia Atrophica (Dystrophia Mytonica) Improved by Testosterone Propionate. *Frederick H. Hesser, Orthello R. Langworthy and Samuel A. Vest* 241
Further Studies on the Purification of Mare Gonadotropic Hormone. *Harold Goss and H. H. Cole* 244
The Sojourn of Insulin in the Blood of Rabbits after the Administration of Massive Doses of Insulin. *Leonard F. Jourdonais and Maurice Bruger* 250
Further Studies on the Relation of the Thyroid and Hypophysis to Ovarian Responses of Menopause Urine Extracts. *S. L. Leonard and J. De Frates* 255
The Mode of Action of Testosterone Propionate on the Female Genital Tract. *John W. Huffman and Louis H. Bos* 259
Obesity in the Rat Following the Injection of Chromic Acid into the Hypophysis. *Albert W. Hetherington* 264
Androgenic Action of Desoxycorticosterone. *Charles W. Hooker and Vincent J. Collins* 269
Experiments on the Biological Properties of Stilbestrol and Stilbestryl Dipropionate. *C. H. Mellish, A. J. Baer and A. C. Macias* 273
The Influence of Estriol, Estradiol and Progesterone on the Secretion of Gonadotropic Hormones in Parabiotic Rats. *Clyde Biddulph, Roland K. Meyer and Laurence G. Gumbreck* 280
Symposium on Carbohydrate Metabolism
Glycogen Breakdown and Synthesis in Animal Tissues. *Carl F. Cori* 285
The Liver and Carbohydrate Metabolism. *Samuel Soskin* 297
The Adrenal Cortex and Carbohydrate Metabolism. *C. N. H. Long, B. Katzin and Edith G. Fry* 309
The Pituitary Gland and Carbohydrate Metabolism. *F. G. Young* 345
Association Notice 352
Current Endocrine Literature 355

NO. 3, MARCH, 1940

The Antigonadotropic Hormones in Normal Human Blood Serum. *Marvel-Dare Fellows* 369
The Effects of Testosterone Propionate on Epiphyseal Closure, Sodium and Chloride Balance and on Sperm Counts. *E. Perry McCullagh and E. J. McGurl* 377
Macrogenitosomia Precox Associated with Hyperplasia of the Androgenic Tissue of the Adrenal and Death from Corticoadrenal Insufficiency. *L. Wilkins, W. Fleischmann and J. E. Howard* 385
Masculinizing and Non-masculinizing Carcinomata of the Cortex of the Adrenal Gland. Report of Six Adult Cases. *Thomas H. McGavack* 396
The Age Distribution and Sex Incidence of Hyperthyroidism and Hypertension. Interrelation of These Two Conditions. *S. K. Robinson* 409
The Endocrine Aspect of Essential Hypertension and Diabetes Mellitus. *James H. Hutton, James T. Case, William L. Culpepper, E. C. Olson and E. E. Madden* 418
Estrogen Metabolism in Cancerous and Non-cancerous Women. *Gregory Pincus and Mark Graubard* 427
Alopecia Totalis. With Special Reference to Familial and Endocrine Factors. *Juliet E. Thorner* .. 433
Hexamine Insulin. Juvenile Type of Diabetes Treated with Hexamethylene Tetramine Insulin Compound Giving Rapid and Sustained Action. *Henry M. Feinblatt, Edgar A. Ferguson and Barnett Alpert* 437
The Increased Duration of Insulin Action by the Use of Protamine Zinc Insulin in Pellet Form. *Jerome Mark and Gerson R. Biskind* 444
The Effect of Testosterone on the Monkey Uterus and the Administration of Steroidal Hormones in the Form of Deanesly-Parkes Pellets. *Carl G. Hartman* 449
The Work Performance of Adrenalectomized Rats Treated with Corticosterone and Chemically Related Compounds. *Dwight J. Ingle* 472
The Work Capacity of the Rat Immediately Following Partial Adrenalectomy. *Dwight J. Ingle* .. 478
On the Mode of Action of Calciferol in the Nephrectomized Rat. *W. R. Tweedy, R. D. Templeton, Mary C. Patras and F. A. McJunkin* 481
The Effect of the Thyrotropic Principle of the Anterior Pituitary on the Thyroid of the Undernourished Guinea Pig. *D. J. Stephens* 485
Liver Growth in Rats Treated with Anterior Pituitary Growth Hormone. *Milton Lee and William Freeman* 493

Effect of a Normal Male Urine Extract on Immature Female Cats. *W. F. Starkey and J. H. Leathem* 499
Observations on Spermatogenesis in Rats. *Eugene Cutuly and Elizabeth C. Cutuly* 503
The Effect of Male Sex Hormone on the Developing Ovaries of Young Fowls. *E. H. Herrick and Charles H. Lockhart* 508
Precocious Copulatory Activity Induced in Male Rats by Subcutaneous Injections of Testosterone Propionate. *Calvin P. Stone* 511
The Recovery of Testes after Androgen Induced Inhibition. *R. R. Greene and M. W. Burrill* 516
The Influence of Temperature on the Internal Secretory Activity of Transplanted Ovaries in the Female Rat. *A. K. Lampton and A. J. Miller* 519
Pharmacodynamic Effects of Testosterone Propionate on Tubal Contractions (Oviduct of Rabbit) Determined by CO_2 Insufflation. *I. C. Rubin and A. M. Davids* 523
Clinical and Laboratory Notes
Preparation of the Gonadotropic Hormone from the Anterior Pituitary of the Sow. *C. Arthur Elden and M. D. Fellows Nutting* 526
Further Studies of the Hormone Content of Human Tissues in Pregnancy. *Frederic Parker, Jr., and Benjamin Tenney, Jr.* 527
The Mechanism and Treatment of Premenstrual Distress with Ammonium Chloride. *J. P. Greenhill and S. C. Freed* 529
Estrogens of the Fowl. *H. W. Marlow and Dan Richert* 531
The Relative Effects of Progesterone and Testosterone Propionate upon Ovipositor Lengthening of the Female Bitterling. *Israel S. Kleiner* 534
The Emptying Time of the Human Stomach after the Administration of Progestin. *Clark K. Sleeth and Edward J. Van Liere* 535
Dysmenorrhea Relieved by Thyroid. *H. Gray* 536
Pituitary Mitotic Changes after the Administration of Estrogen and after Ovariectomy. *W. C. Kuzell and W. C. Cutting* 537
Current Endocrine Literature 539

NO. 4, APRIL, 1940

Quantitative Studies on the Hormones of Human Pituitaries. *Emil Witschi and Gardner M. Riley* 565
Antibody Response in Rabbits to Extracts of Human Pregnancy Urine and to Extracts of Normal Female Urine. *Katharine M. Howell and Samuel Soskin* 577
The Action of Diethylstilbestrol in Gynecological Dysfunctions. *Raphael Kurzrok, Leo Wilson and William H. Perloff* 581
The Action of Estradiol, Progesterone and Testosterone on the Contractions of the Human Uterus. *Leo Wilson and Raphael Kurzrok* 587
Sex Hormone Studies in Male Homosexuality. *S. J. Glass, H. J. Deuel and C. A. Wright* 590
The Effect of Anterior Pituitary-Like Sex Hormone on the Blood Picture in Man. *William M. Moffat* 595
Endocrine Dwarfism. *Robert L. Schaefer and Fred L. Strickroot* 599
Pituitary Function in Adiposogenital Dystrophy (Fröhlich). *Herman Schwarz, Albert B. Newman and Harry Baum* 605
Clinical Method for Estimating Monthly Output of Estrin from Single Bioassay on Pooled Night Specimens of Urine. *John Lansbury and Joseph Hughes* 609
Effects of Large Doses of Protamine Zinc Insulin in Non-Diabetic Individuals. *Douglas Goldman* 612
Metabolism of the Estrogens. The Effect of Liver and Uterus upon Estrone, Estradiol and Estriol. *Carl G. Heller* 619
Fluid and Electrolyte Shifts in the Normal and Adrenalectomized Rat after the Intraperitoneal Injection of Isotonic Sugar Solutions. *John W. Remington* 631
Blood Adrenalin Levels During Insulin Shock Treatment for Schizophrenia. *Esther Bogen Tietz, Harry Dornheggen, and Douglas Goldman* 641
Further Studies on the Andromimetic Function of the Immature Male Rat Adrenal. *M. W. Burrill and R. R. Greene* 645
Variations in the Effectiveness of Percutaneously Applied Androgens in the Rat. *Dorothy Nelson, R. R. Greene and J. A. Wells* 651
Studies on the Inhibitory Hormone of the Testes. IV. Effect on the Pituitary, Thyroid and Adrenal Glands of the Adult Male Rat. *Ben Vidgoff and Herman Vehrs* 656
The Effects of Testosterone Propionate on the Ovariectomized Mature Rat. *Milton Mazer and Charles Mazer* 662
Adolescence of Macaques. *Ruth M. Smith and Boris B. Rubenstein* 667

Influence of the Uterus on the Corpus Luteum. *Oscar Hechter, Max Fraenkel, Maurice Lev and Samuel Soskin* 680
The Reponse of Rat Uteri to Hormone Injections. *Gregory Pincus and Mark Graubard* 684
The Effect of Stilbestrol on the Basal Metabolism of Experimental Hyperthyroid Rats. *T. C. Sherwood* 693
The Metabolism of the Thyrotrophic and Gonadotrophic Hormones. *S. M. Seidlin* 696
The Effect of Activated Ergosterol and of Parathyroid Hormone on Gastric Secretion in the Dog. *B. P. Babkin, Olga Komarov and S. A. Komarov* 703
A Comparison of Methods of Assay of the Lactogenic Hormone. *A. J. Bergman, J. Meites and C. W. Turner* 716
The Lipoid, Calcium, Phosphorus and Iron Content of Rats with Hypothalamic and Hypophyseal Damage. *A. W. Hetherington and Arthur Weil* 723
Current Endocrine Literature 728

NO. 5, MAY, 1940

Effect of Human Diabetic Plasma upon Blood Sugar Curves in Rabbits Following Insulin. *Alexander Marble, Alison T. Fernald and Rachel M. Smith* 735
Reverse or 'Paradoxical' Blood Sugar Reponse to Ingested Glucose. A Correlation of Laboratory and Clinical Findings in Forty Patients. *Homer Wheelon* 743
Bioassays for Urinary Androgens. A Comparison of the Results in Normal Men with Those Having Endocrine Disturbances. *E. P. McCullagh and H. V. Lilga* 753
Percutaneous Administration of Testosterone Propionate for Dysmenorrhea. *A. R. Abarbanel* 765
Maintenance of the Corpus Luteum Function in Women by Estrogenic Substances. *Axel Westman* 774
The Assessment of Skeletal Development of the Hand and Knee Between the Ages of 8 and 14 years. *Leona M. Bayer and Robert R. Newell* 779
Thyroid Gland Disturbance as a Cause of Unexplained Fever. *Robert C. Moehlig* 783
A Clinical Classification of Hypothyroidism. *Harry A. Solomon, Arthur Weiss and Elliot Hochstein* 785
Report of Three Cases of "Clinical" Addison's Disease Surviving More Than Fifteen Years. *L. G. Rountree* 793
Bilateral Localized Lesions in the Hypothalamus with Complete Destruction of the Neurohypophysis in a Pituitary Dwarf with Severe Permanent Diabetes Insipidus. *A. B. Baker and Charles B. Craft* 801
Benzedrine Sulfate in Obesity. *Gerhard Rosenthal and Harry A. Solomon* 807
The Comparative Action of Graded Doses of International Standard Gonadotropic Substance (Chorionic Gonadotrophin) on Normal Infantile Male and Female Rats. *J. Leon Sealey and C. W. Sondern* 813
The Relation of the Anterior Pituitary to Bile Production. *O. B. Houchin and C. W. Turner* 821
Biological Indicators of Androgenic Activity. *Dorothy Nelson and R. R. Greene* 824
The Action of Trasentin on the Rabbit Ovary and Uterus. *Raphael Kurzrok, Edgar G. Miller, Jr., Harry Gegerson and Alan Gegerson* 827
The Effect of Estriol Glucuronide on the Spontaneous Activity of Senile Male Rats. *R. G. Hoskins and Sylvia Bevin* 829
Inhibition of Pelvic Changes Occurring During Pregnancy in Mice by Testosterone Propionate. *W. U. Gardner and J. Van Heuverswyn* 833
The Effect of Testosterone Propionate on the Black-Crowned Night Heron. *G. K. Noble and M. Wurm* 837
The Synergistic Action of Thyroid on Fluorine Toxicity. *Robert H. Wilson and Floyd DeEds* 851
Cytological Evidence of Secretory Activity in the Mammalian Parathyroid Gland. *Allan L. Grafflin* 857
Growth of Vitamin Deficient Rats Treated with Thymocrescin. *Albert Segaloff and Warren O. Nelson* 860
An Attempt to Produce Increased Susceptibility to Renal Ischemia in Rabbits by Means of Endocrine Preparations. *L. V. Dill and C. E. Isenhour* 863
Cortin and the Na Factor of the Adrenal. *F. A. Hartman and H. J. Spoor* 871
Further Studies on the Refractory State Developed Following Repeated Injections of Adrenal Extract. *Frank A. Hartman, Lena A. Lewis and Jane E. Gabriel* 879
Compensatory Hypertrophy of the Dog Adrenal Gland Following Unilateral Adrenalectomy. *C. A. Swinyard and H. D. Bruner* 886
The Reaction to Pituitrin and Adrenalin of the Myometrium of the Domestic Sow. *Elijah Adams* 891

The Cell Contents of the Cortex of the Suprarenal Gland. *Marjorie V. N. Sudds* 895
The Effect of Adrenalectomy on the Ketosis Produced in Rats by Anterior Pituitary Extract. *Reginald A. Shipley* 900
Editorial Article
The Endocrine Literature of 1939. *Herbert M. Evans and Barbara Cowles* 906
Clinical and Laboratory Notes
Effect of Extracts of Gelding Urine on Reproductive System of the Rat. *Charles E. Rea* 913
Pregnancy Diagnosis in Mares by Colorimetric Estimation of Estrogens. *Salomon Kober* ... 914
Current Endocrine Literature 916

NO. 6, JUNE, 1940

Adrenal Cortical Virilism. *F. D. W. Lukens and H. D. Palmer* 941
Estrogenic Effects upon Tubal Contractility and the Vaginal Secretion in the Menopause. Study of Twenty-four Cases with the Aid of Uterotubal Insufflation. *Phineas Bernstein and Morris Feresten* 946
Testosterone Propionate and the Male Climacteric. *H. B. Thomas and R. T. Hill* 953
Menstruation and the Endometrium. *Leo Wilson and Raphael Kurzrok* 955
Determination of PH of Vaginal Secretion as an Index of Ovarian Activity in Hypoovarian States. *Jacob S. Beilly* 959
Hormone Excretion and Bioassay of Extirpated Tumor in Teratoma of the Testis. *Jacob S. Beilly, Irving Kurland and Mendel Jacobi* 965
Effects of Anterior Pituitary Extracts in the Fasted Rat. *Helen C. Harrison and C. N. H. Long* .. 971
Experimental Studies of the Anterior Pituitary. V. Functional Activity of Anterior Pituitary Grafts in the Adult Male Guinea Pig. *M. Schweizer, H. A. Charipper and W. Kleinberg* .. 979
Photoelectric Plethysmography of the Female Breast. *Sam. Gordon Berkow and Murray B. Jacobson* 986
Fractionation of Saline Extracts of Anterior Pituitary Tissue. *Roy. W. Bonsnes and Abraham White* 990
Studies in the Physical Chemistry of the Anterior Pituitary Hormones. *H. L. Fevold, Milton Lee, F. L. Hisaw and E. J. Cohn* 999
The Comparative Assay of Gonadotropic Substances on Rats, Mice and Chicks. *John S. Evans, Leonard Hines, Roger Varney and F. C. Koch* 1005
Comparative Study on the Augmentation of the Action of Gonadotropins from Various Sources in the Rat, Mouse and Chick. *John S. Evans, Leonard R. Hines, Joseph J. Ceithaml and F. C. Koch* 1012
Physiological Significance and Morphology of the Carmine Cell in the Cat's Anterior Pituitary. *H. B. Friedgood and A. B. Dawson* 1022
The Relationship Between the Secretory Cells of the Pars Nervosa of the Hypophysis and Classical Neuroglia. *Mervyn Griffiths* 1032
The Effect of Certain Experimental Conditions upon the Thyrotropic Hormone Content of the Albino Rat. *C. W. Turner and P. T. Cupps* 1042
The Effects of Ovariectomy and Administration of Progesterone on the Adrenal X-Zone and the Uterus. *Evelyn Howard and Saiyude Gengradom* 1048
Thyrotropic Hormone and Fluorine Activity. *Floyd DeEds, Robert H. Wilson and Windsor C. Cutting* 1053
The Effects of Antuitrin-S, Progestin and a Vital Dye on Late Pregnancy in the Rat. *Russell M. Coco* 1057
Creatine-Creatinine Metabolism and the Hormones. III. Effect of Parenteral Injection of Creatine and Creatinine with the Sex Hormones upon Creatine-Creatinine Excretion in Normal Animals. *Howard H. Beard and Ernest J. Jacob* 1064
Creatine-Creatinine Metabolism and the Hormones. IV. Effect of Parenteral Injection of the Sex Hormones, with and without Saline and Creatinine upon Creatine-Creatinine Excretion in the 90-day Castrate Rat. *Howard H. Beard and Ernest J. Jacob* 1069
The Effect of Multiple Injections of Progesterone on the Turgescent Perineum of the Baboon (*Papio Porcarius*). *Joseph Gillman* 1072
The Effect of Testosterone, Estrone and Estradiol Applied Locally to the Penis of the Rat. *H. S. Wigodsky and R. R. Greene* 1078
Effects of Testosterone Propionate on Pregnancy and on Passage of Ova Through the Oviducts of Mice. *H. O. Burdick, Basil B. Emerson and Rae Whitney* 1081
Urolithiasis in the Rat after Injection of Testosterone Propionate. *J. Gershon-Cohen, Harry Shay, Karl E. Paschkis and Samuel S. Fels* 1087

Limitation of Food Consumption as a Factor Influencing Endocrine Reactions in the Chick.
W. R. Breneman 1091
Association Notice 1099
Current Endocrine Literature 1103
Index 1115

Errata, Volume 25

Page 946, line 26. For demobilized *read* mobilized.
Page 948, line 7. For pure *read* purified.
Page 949, line 8. For Rats 4949 and 5468 *read* Rats 4949, 5468 and 5469.
Page 949, last line. For 0.12 *read* 0.13.
Page 951, heading of table 7, last sentence. Read The zone of action is from 0.06 to 0.000002 Rb. U.
Page 952, line 3. For Prolution *read* Proluton.
Page 952, reference 1. Read WINTER, E. W.: Arch. f. Gynäk. 141: 588. 1930.

Volume 26

Page 87, reference 2. For "S. Afr. J. M. Sci." *read* "S. Afr. J. Sci."
Page 525, line 21. For Hoffman La-Roche, Inc., *read* Roche-Organon, Inc.

ENDOCRINOLOGY

THE BULLETIN OF
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VOLUME 26 JANUARY, 1940 NUMBER 1

ACROMEGALY AND DIABETES MELLITUS

CHESTER COGGESHALL AND HOWARD F. ROOT

From the George F. Baker Clinic,[1] New England Deaconess Hospital
BOSTON, MASSACHUSETTS

SINCE THE FIRST description of acromegaly by Pierre Marie, repeated clinical observations and a large series of experiments have demonstrated both that the pituitary gland possesses the power of influencing carbohydrate metabolism in the general sense and that in cases of hyperpituitarism, best illustrated by acromegaly, diabetes develops with unusual frequency. The demonstration that extracts of the anterior pituitary when given by injection in large amounts will produce diabetes in a dog has led to the supposition that a disorder of the pituitary gland might be the underlying cause for most if not all cases of diabetes mellitus. In order to test this hypothesis not only clinical but pathologic evidence would be necessary. It is well known that one may have a clinical syndrome without uniform and demonstrable structural changes. Such might be the case with the relation between the pituitary gland and diabetes. It might explain the small proportion of diabetic cases without evident changes in the pancreas. Indeed, since Young (1) and subsequently Campbell and Best (2) have shown that injections of relatively large amounts of an extract obtained from the anterior pituitary gland can cause varying degrees of degenerative change and final destruction of the islands of Langerhans in a dog, a pituitary disorder characterized by hypersecretion of some substance by the anterior portion of the pituitary might underlie the cases of diabetes mellitus in which there are easily demonstrable changes in the pancreas. One must recognize the fact, however, that in diabetes pathologic change in the pancreas is found in fully 73% of the patients, and there are associated pathologic changes in the liver, kidneys and arteries in patients who have had diabetes a sufficient length of time for the pathologic picture to become fairly definite. The same is particularly true of acromegaly, the one clinical condition in which overactivity of the acidophilic cells of the anterior pituitary is definitely recognized as the cause of symptoms. The substance or hormone which causes the overgrowth in acromegaly is a product of these acidophilic cells. The wide variety of changes in the various tissues of the body is due to the extra secretion of this substance and the clinico-pathologic picture is definite.

We are here reporting observations upon: *a*), 26 cases of diabetes occurring among 153 acromegalic patients studied on the service of Dr. Harvey Cushing at the Peter Bent Brigham Hospital, Boston; *b*), evidences of hyperpituitarism among diabetic patients at the George F. Baker Clinic, New England Deaconess Hospital, Boston and *c*), 3 frank cases of acromegaly and 3 of gigantism at the George F. Baker Clinic.

[1] Elliott P. Joslin, Medical Director.

TABLE I. 26 CASES OF DIABETES MELLITUS AMONG 153 PATIENTS WITH ACROMEGALY[1]

PBBH case number	Sex	Hebrew	History of diabetes in family	Age at onset of		Interval between onsets	Duration to death or last report		Treatment before onset of diabetes	Living or dead	Autopsy	Cause of death	Clinical character of diabetes
				Acromegaly	Diabetes		Acromegaly	Diabetes					
26828	F	yes	o	35	46	11	18	7	Surgery	D	o	Diabetes	Insulin reduced glycosuria, constant hyperglycemia.
33384	F	yes	o	29	39	10	18	8		D	o	Diabetic infection	Insulin and diet controlled glycosuria, cyclic hyperglycemia.
61	M	no	o	20	33	13	15	2		D	+	Cardiac	Pre-insulin era. Diet controlled glycosuria somewhat.
589	M	no	o	26	34	8	17	9		D	+	Intestinal hemorrhages	Pre-insulin era. Cyclic glycosuria on hospital diet.
3625	F	no	o	30	31	21	22	1	Surgery	D	+	Diabetic coma, broncho-pneumonia	Pre-insulin era. Unrestricted CHO diet.
20016	F	no	o	22	24	2	3	1	Surgery	D	o	Diabetic coma, infection	Pre-insulin era. Untreated diabetes.
19778	M	no	+	29	39	10	25	15	X-ray	L		(alive in 1938)	No insulin. Mild glycosuria. Possibly disappearance of diabetes
27528	F	?	+	19	32	13	20	7		L		(alive in 1932)	Cyclic glucose tolerance curves and glycosuria.
41058	F	no	o	20	28	8	11	3	X-ray	D	o	Cerebral tumor, ? of metastases	Untreated diabetes. Constant glycosuria.
17436	F	?	o	23	32	9	21	12	X-ray	L		(alive in 1938)	Diet controlled glycosuria and acidosis but not hyperglycemia.
10035	F	yes	o	26	41	15	25	10		L		(alive in 1934)	Hyperglycemia and glycosuria responded to insulin and diet.
27099	F	no	+	19	21	2	11	9		L		(alive in 1934)	Mild diabetes, having no insulin or diet therapy.
720	F	no	o	18	34	16	22	6		D	o		Pre-insulin era. No diabetic treatment.
29322	F	no	o	24	43	19	25	6	Surgery	D	9		Reaction once with insulin. Lugolization 'cured' diabetes.
27726	F	no	+	49?	50	1?		(probably dead)				?	Glycosuria apparently paralleled hyperglycemia.
951	F	yes	o	20	35	15	22	7	Surgery	D	o	Carcinomatosis	Mild glycosuria and hyperglycemia untreated.
32347	M	no	o	31	35	4	16	12		D	o	Diabetes, influenza	Perhaps insulin-sensitive. Probably diabetic coma.
15505	F	no	o	44?	56	12	16	4		D	o	Diabetes, coma, vascular disease	Insulin with unrestricted diet had little effect.
1140	M	no	o	27	43	16	24	9		D	o	Endothelial meningioma	Diabetes responding well to diet therapy.
19066	M	yes	o	36	42	6	23	17		D	+	Cor. thrombosis, dissect. aneurysm	Cyclic hyperglycemia, glycosuria and insulin sensitivity.
36317	F	no	o	45?	50	5		(probably dead)				?	Diabetes responding well to diet and insulin.
4215	M	yes	o	44?	45	1?	14	13		D	o	Hypernephroma, rupt. pit. cyst	No signs of acidosis even with urine sugars of 5%.
20989	M	no	o	19	22	3	3	0	Surgery	D	o	basal brain tumor	Glycosuria responded to diet. ? hypoglycemia attacks.
22075	F	no	o	34	39	5	18	13		L		(alive in 1930)	Diabetes well controlled by diet and insulin.
41058	F	no	o	20	29	9	10	1	Surgery	D	o	Diabetes	Insufficient data.
36745	F	yes	+	41	56	15	25	5	X-ray	L			Mild with diet and insulin.
Total 26	8 M 18 F	27%	19%	28.9 yrs.	38 yrs.	9.5 yrs.	17.6 yrs.	7.5 yrs.			4	7 from diabetes— 3 from coma	

[1] These 153 cases of acromegaly were observed at the Peter Bent Brigham Hospital between 1913 and 1932.

INCIDENCE OF DIABETES MELLITUS AMONG ACROMEGALICS

In 1927, Cushing and Davidoff (3) reported that diabetes mellitus occurred in 12% of a group of 100 individuals having acromegaly. We have reviewed their original 100 cases plus 53 cases of acromegaly subsequently studied at the Peter Bent Brigham Hospital, with special attention to the development of diabetes mellitus. By a follow-up method[2] a total of 26 cases of diabetes (table 1) were discovered in the 153 acromegalics, an incidence of 17.0%. Likewise their estimate of the incidence of glycosuria as 25% was augmented by our findings to 36%.

Since acromegaly is a progressive disease, usually of long duration, the time interval between the onset of acromegaly and the onset of diabetes might indicate to some degree the influence of hyperpituitarism as an etiologic factor in diabetes. A definite demonstration of such a relation might be found if study were given to those who had received effective treatment of the hyperpituitarism in contrast to those not so treated. Evaluation of treatment, however, was difficult since cases presenting signs of intracranial pressure were subjected to surgery with its attendant higher mortality while others were given only irradiation therapy. Moreover, since acromegaly is characteristically cyclic in its intensity, the time interval alone cannot represent fairly the intensity of the hyperpituitarism. The average interval between the onset of acromegaly and that of diabetes was 9.5 years but the actual intervals varied from 1 to 22 years with the majority of cases of diabetes occurring within 15 years. That treatment of the acromegaly had no clear-cut effect upon the incidence of diabetes other than the prolongation of acromegalic life is brought out by the fact that the intervals between the onsets of the two diseases in the 10 cases having some therapy directed towards the pituitary gland showed the same wide variations as did those in the untreated group. The average duration of life of the acromegalics in whom diabetes did not develop was 13.1 years. We believe this figure important since it indicates that most of these patients lived long enough to develop diabetes.

The preponderance of females over males developing diabetes in these acromegalics is of some interest since Davidoff and Cushing noted an almost equal sex incidence in acromegaly and furthermore since Joslin (4) has pointed out that since 1922 the proportion of female to male diabetics has been increasing. There were 8 males and 18 females. The average age at onset of the diabetes was remarkably similar for both sexes, 38.8 years. This age differs strikingly from the median age at onset of 6357 diabetics (5) for whom it was 46.5 years for males and 49.2 for females. The durations of the diabetes showed a wide variation from 0 to 17 years with an average of 8.6 years. This is not inconsistent with the average expectation of life for all diabetics for this period. As will be pointed out later, the use of insulin was apparently of far less importance in the prolongation of life in these acromegalic diabetics than it was in non-acromegalic diabetics. Hebrews numbered 27% of the acromegalic series in contrast to 6357 diabetics (5) among whom Jewish males constituted 11.6% of all males, and Jewish females 16.3% of all females. An hereditary factor was frankly evident in only 5 of the 26 diabetics. No particular effort had been made to elicit a family history of diabetes in the earlier series, nor has the influence of repeated questioning been adequately ascertained, owing to deaths of patients. Thus *Case 27099* denied the existence of diabetes in her family in 1926, but in 1938 brought in two aunts for treatment of diabetes! Careful follow-up might show that even acromegaly only produces diabetes in those with an hereditary predisposition.

[2] Through the courtesy of the late Professor Harvey Cushing and the Peter Bent Brigham Hospital, the records of these patients were made available for study and follow-up. Grateful acknowledgement is made here.

Diabetic symptomatology was difficult to evaluate and often the date of onset was uncertain. The onsets given in the tables represent the first laboratory evidence of hyperglycemia associated with glycosuria. Such common symptoms as polyuria, polydipsia and polyphagia were frequent enough but are as much a part of acromegaly as of diabetes. Loss of weight after the period of abnormal growth, a marked intensification of the polyuria and polydipsia, and pruritus vulvae often marked the true onset of the diabetes.

The clinical course of the diabetes varied greatly from an apparently incidental and innocuous finding to severe diabetes ultimately causing death. This is discussed in detail in the analyses of the individual diabetic cases. Three of the 16 cases reported dead, died of diabetic coma and it is probable that diabetes per se was more than a contributing cause in 3 more cases.

In the 26 cases of 'acromegalic diabetes' 12 individuals displayed no evidence of sterility although it must be admitted that most of the children of these patients were born in the early years after the onset of acromegaly. Apparently the secondary glandular effect on the generative organs, like the onset of diabetes, occurs later in the course of the acromegaly. Miscarriages were noted in 4 patients before the onset of diabetes mellitus and this is of particular interest since their occurrence is today a major problem during the pregnancies of diabetic patients.

The common complications of diabetes were frequent in this series. Arteriosclerosis and pyogenic infections were both noted in 6 of the 26 cases. Thyroid enlargement, usually an adenoma, was a common finding. *Case 33384* had a thyroidectomy without apparent effect on the diabetic manifestations. Her B.M.R. was +74% at the time of the thyroidectomy. *Case 20016* was unusual in that she gave a history of primary amenorrhea, had a B.M.R. of −23% and yet died of diabetic coma complicating an infection. An abnormal liberation of thyrotropic hormone may well have caused the thyroid adenomata.

Dietary management, because of the remarkable polyphagia attendant upon the acromegaly, was seemingly without success in most of these patients. There was little restriction of carbohydrates except when the patients were hospitalized. In general it can be said that the mere restriction of carbohydrates had little or no effect on the heights of the fasting blood sugars in most cases. In fact, a normal blood sugar was a rarity.

An answer to the question as to whether or not the anterior lobe of the pituitary secretes a hormone or hormones antagonistic to insulin has been sought for in these cases of acromegaly and diabetes. An increased resistance to insulin was noted by several workers (3, 6–11). The action of insulin was thought to be normal by many others (12–22).

Ulrich (11) described a man 24 years of age in whom a pituitary tumor was discovered on July 3, 1924. Glycosuria was absent. On December 13, 1924 sugar in the urine amounted to 238 gm. in 24 hours. During the next few days insulin dosage was gradually increased to 360 U a day because of a blood sugar rising from 404 to 625 mg. Within a month so rapid was the disappearance of glycosuria and fall of the blood sugar that his urine became sugar free without the use of any insulin and with a non-diabetic diet. Six months later, following operation upon the tumor, insulin was again used to reduce a blood sugar which was described as a traumatic hyperglycemia due to surgical trauma in the neighborhood of the pituitary. In November, 1926 he returned and again required insulin which for a brief period reached 100 U a day and was finally given up. He died not long after and at autopsy a large pituitary adenoma was found. This was clearly a case in which the severity of the diabetes associated with acromegaly fluctuated greatly, and indeed it may be argued that the rapid change in the severity of the diabetes was due solely to change in anterior pituitary activity and was completely unaffected by the insulin or diet. We hold, however, that it is possible that the excessive pituitary secretion was checked by the use of insulin. Actually the results ob-

tained in this case during 3 or 4 weeks by use of insulin and diet were much better than are often obtained in patients showing ordinary diabetes so far as urine sugar and blood sugar are concerned. The fact that the patient showed less fall in the blood sugar with 100 U than another patient with ordinary diabetes with only 50 U, merely shows a difference in the degree of sensitiveness to insulin such as can be frequently demonstrated in any group of diabetic patients who do not have pituitary disease. The diabetes did not disappear but merely became very mild. Such transitory insulin resistance is unlike genuinely severe resistance persisting for periods of many months in spite of careful dietary control. Such resistance has rarely been associated with pituitary disorders. Thus the physician who eventually required 1600 U of insulin a day (23) had hemochromatosis at autopsy. *Case 14483*, a nurse (24) who required 500 U a day while undergoing bed treatment in the Deaconess Hospital for 6 months had rheumatoid arthritis. Recently a man, (*17269*) 33 years of age, has taken 200 to 300 U daily but shows no evidence of endocrine disorders or foci of infection.

Buchanan and Indelicato (25) cite a case of xanthoma diabeticorum associated with diabetes and acromegaly in which large daily doses of insulin failed to control the hyperglycemia or glycosuria. After 4 years observation, during which time blood sugars were repeatedly high, the patient is still alive although he is taking 150 to 160 U of insulin each day.

Blum and Schwab (12), however, gave 30 U of insulin to each of 2 patients, one who was suffering from acromegalic glycosuria and the other from pancreatic diabetes, and found the responses essentially equal.

TABLE 2. GLUCOSE TOLERANCE CURVES IN NORMAL, MILD DIABETICS AND ACROMEGALICS WITH MODERATE DIABETES

	Blood Sugar		
	Normal	Mild Diabetes	Acromegalics with Diabetes
	mg. %	mg. %	mg. %
Fasting	90	95	210
100 gm. glucose			
½ hour	150	180	238
1 hour	110	200	310
1½ hours	100	175	315
2 hours	85	150	318

Campbell (26) states that "in both the acidophil and basophil adenomata, glycosuria and diabetes are periodically present and, curiously enough, are apparently not readily regulated by insulin." Hetzel (15) insisted that the glycosuria in a case of acromegaly was identical with that of diabetes and reacted similarly to insulin and the control of diet. Our view that 'pituitary' diabetes does not differ from pancreatic diabetes in its response to insulin therapy is shared by John (27).

The mild cyclic nature of acromegaly and the apparent lack of correlation between the height of the blood sugar and the amount of urine sugar makes the problem a difficult one to settle. Table 2 shows a comparison of glucose tolerance curves in normals, mild diabetics and acromegalics with moderately severe diabetes. Many of these 26 cases (table 1) were studied before the discovery of insulin; others, though given insulin, were not adequately managed in regard to diet. The first 2 cases were studied also at the New England Deaconess Hospital. *Case 12* was subsequently studied by one of us.

Case 1 (*5447*), was a married Hebrew woman, age 48. Her height was 158.8 cm., weight 73 kg. She was referred by Dr. Harvey Cushing for diabetic study on August 2, 1926. Bitemporal and frontal headaches had been noted since 1913 with an increase in the size of the hands and feet and a noticeable coarseness of the facial features since 1917. Vision became markedly blurred and a decompression craniotomy had been performed in 1921. Two opera-

tions were performed by Dr. Cushing in 1921 and 1925. Sugar was first discovered in the urine in January, 1925 following a noticeable exaggeration of symptoms of polyphagia, polyuria and polydipsia with a loss of weight during the previous 6 months.

On admission the urine contained 2.0% sugar and the blood sugar was 270 mg.%. On an average daily diet containing carbohydrate, 88 gm., protein, 35 gm. and fat, 57 gm. for 3 days, there was a reduction in the fasting blood sugar to 230 mg.% and the urinary sugar became 1.0%. The patient was placed on a diet of C 150, P 60 and F 90, and insulin therapy begun. When the insulin was reduced from a daily dose of 25 U to 6 U, urinary sugar increased from 5 gm. to 10, 8 and 26 gm. on 3 successive days. When insulin was resumed it dropped successively to 12, 2 and 6 gm. Blood sugars fasting continued to be high throughout this insulin manipulation (270, 230 and 270 mg.%). The B.M.R. was +31% and +33% on 2 days. An increase in the carbohydrate to 165 gm. made no difference in the urinary sugar percentage or fasting blood sugars. She died of diabetes in 1931.

An acromegalic diabetic, who was apparently sensitive to changes in insulin dosage (i.e., increase in insulin caused a drop in the urinary sugar content) gave evidence of but slight effect upon blood sugar values and general diabetic status by a diabetic diet.

Case 2 (4219), a married Hebrew woman, age 40, height 161.3 cm., weight 66.5 kg., was first seen on October 4, 1924. The onset of acromegaly apparently was in 1913 when the patient noticed that her nose and feet were becoming larger. On October 13, 1923 she was admitted to the Peter Bent Brigham Hospital on the service of Dr. Harvey Cushing. Urine contained 2.5% sugar, the blood sugar fasting value was 280 mg.%. On a daily diet containing small amounts of carbohydrate (33, 77 and 118 gm.) the fasting blood sugar was between 130 and 150 mg.%. A glucose tolerance test at this time gave the following blood sugar values, fasting, 130; ½ hour, 200; 1 hour, 150; 2 hours, 120. No operation on the pituitary gland was attempted.

On admission to the George F. Baker Clinic October 4, 1924, urine sugar was 6.5% with a non-fasting blood sugar of 310 mg.% and a CO_2 combining power of 51. On an average daily diet containing carbohydrate 57 gm., protein 36 gm. and fat 66 gm., three successive days with insulin 15 U daily, the urine sugar fell to 0.9% but the fasting blood sugar remained high (320 mg.). The diet was increased to average C 80, P 65, F 150, and the urine became sugar free although fasting blood sugar values ranged from 170 to 250 mg.%. This diabetic picture continued over the initial 40-day observation, even though during the last 11 days the patient refused to take her insulin and was on a diet of C 90, P 75 and F 150 with a suspected carbohydrate addendum of toast from other patients' trays. In August of 1928, a nodular thyroid gland (B.M.R. +74%) was removed by Dr. Frank Lahey. In September of 1929, she was again admitted for diabetic observation and for treatment of an infected ulcer of the left leg with lymphangitis extending to the groin. Urine sugar was 8.6%, blood sugar 390 mg.%. On a diet approximating C 110, P 60, F 90, with an average of 70 U of insulin a day, the urine became sugar free and the blood sugar fell to 160 mg. 4 hours after breakfast.

On November 1, 1928, she was readmitted because of an abscess of the right leg. The urine contained 5.5% sugar, the fasting blood sugar was 380 mg.%. On a diet averaging C 160, P 75, F 125, with insulin 25 U, the urine again became sugar free although the blood sugar remained consistently high. On two days when the blood sugars were 320 and 240 mg.% the 24-hour urine samples were sugar free. She was seen again in April and July of 1930 and in February of 1931, each time showing a high urinary sugar percentage. It was apparent that the patient made no attempt to follow a diet at home. She died September 19, 1931 with an infected foot.

The patient was an acromegalic diabetic whose glycosuria responded well to dietary and insulin management. The increase in the severity of this patient's diabetes during episodes of infection seems identical with the reactions of all diabetics to infection.

The other cases are reported much more briefly since we have had no personal experience with their diabetes.

Case 3 (61), an acromegalic diabetic of the pre-insulin era, showed a high percentage of urine sugar on unlimited diets but glycosuria was apparently amenable to 'diabetic sugar-free diets' of an average caloric value of 3000. The urine sugar disappeared during the last few days when he was dying of cardiac failure. (See table 7, *Case* VIII for autopsy.)

Case 4 (589), another acromegalic diabetic before insulin, had urinary sugar ranging from 0.9 to 2.6% while in the hospital. The carbohydrate intake was particularly large. (See table 7, *Case* XI for autopsy.)

Case 5 (3625) died January 25, 1916 in diabetic coma. The patient was on an unrestricted high caloric diet and lived only 1 month after complaining of an exaggeration of her polyuria and polydipsia, and an intense pruritus vulvae. We believe that a terminal bronchopneumonia was of more primary importance in the cause of death than was the diabetic coma. (See table 7, *Case* IX for autopsy.)

Case 6 (20016), a pre-insulin case, died in diabetic coma May 11, 1922 with a complication causing a temperature of 104° F. and an increase in respirations to 50 per minute. The CO_2 was 19 volumes%. Three years before she had had a transphenoidal operation by Dr. Cushing and had apparently developed diabetes 2 years after the operation. The diabetes was untreated. It is interesting to note the existence of a primary amenorrhea in this case.

Case 7 (19778), an acromegalic, was first seen by Dr. Cushing September 28, 1923. In January 1926, the patient wrote, "I have too much sugar in my blood and eat no sweets." In February of 1938, his physician wrote that the patient had been returning for years for deep x-ray therapy. In February of 1923, his glucose tolerance test result was fasting 133 mg.%; and 2 hours after, 265 mg.%. No symptoms of acidosis were experienced and apparently his glycosuria was mild. A non-fasting blood sugar on February 18, 1938 was 130.7 mg.%. The patient's father had died of diabetes. It is possible that this patient had a diabetes which subsequently disappeared as in the cases noted by John (28).

Case 8 (27528), presented two widely varying results after glucose tolerance tests. In March of 1925, the glucose tolerance test results with 100 gm. dextrose was 181, 333, 250 and 243 mg.% with no glycosuria. In November of 1926, with 0.5 gm. of glucose per kg. of body weight the curve was 110, 160, 80 and 100 mg.%; 0.65% of sugar was found in the urine after the test! Apparently the acromegaly was exceedingly cyclic.

Case 9 (41058), an acromegalic diabetic, constantly showed sugar in the urine. She apparently neither used insulin nor restricted her diet. Results of a glucose tolerance test with 100 gm. of glucose on May 13, 1932 were, fasting, 150; ½ hour, 300; 1 hour, 310; 1½ hours, 270; 2 hours, 270. Five days later the test was repeated and the results were 230, 330, 340 and 400 mg.%. The urine sugars ranged from 0.4 to 2.8%.

Case 10 (17436), wrote in 1927: "I have taken another ailment, 'The Sugar Diabetes'," — this after 10 years of acromegaly. In 1930, she was operated upon for multiple abscesses of the breast. Fasting blood sugars ranged from 348, 308 and 453 mg. on the first 3 days of hospitalization, to 294, 267 and 187 mg. on the last 3 days. There was a heavy reduction of sugar with a heavy trace of acetone accompanying the first group of blood sugars while during the last 3 days after the subsidence of the infection no sugar or acetone was found. This case is similar to most cases of diabetes in its reaction to infection.

Case 11 (10035) after having acromegaly for 15 years wrote that she had diabetes: "have had insulin several times but get little results even though I watch my diet closely. Have lost 40 lb." Her physician in 1934 wrote, "Patient weak and sleepy. Metabolism plus 2½ per cent. Urine 1.0 per cent sugar. Eight months ago blood sugar was 250, urine 0. Put on alkaline and diabetic diet with 30 to 50 units of insulin a day. After two weeks, blood sugar 120. Three months later blood sugar 235. Treated at home with same diet and 40 to 80 units of insulin per day. After two weeks of treatment blood sugar was 190 and patient refused to take more insulin." Hyperglycemia seemed to respond to insulin and dietary management.

Case 12 (27099) apparently had a very mild diabetes requiring no dietary or insulin regulation. She was markedly obese (114 kg.). Blood sugars ranged from 100 to 240 mg.%, glycosuria from 0.5 to 1.0%.

Case 13 (720) occurring in the pre-insulin era, died in 1918, never having had any treatment for her diabetes. The blood sugar on one occasion was 270 mg.% and urine sugars ranged from 1.7 to 2.6% with 3 + acetone.

Case 14 (29322) after having had acromegaly for 19 years, developed diabetes. A transphenoidal operation in 1914 apparently arrested the hyperpituitarism for in 1927 her symptoms seemed to be due almost entirely to her diabetes. One of us (H.F.R.) found her urine sugar to range between 8 and 13% and noted a reaction after a small dose of insulin. She was placed on Lugol's solution and the basal metabolism fell from +77% to +24%, the urine becoming sugar free and the blood sugar falling from 220 mg.% to from 70 to 110 mg.% fasting. Control of the hyperthyroidism in this case apparently removed all the findings of diabetes. This case is similar to the one described by Lammli (29) who found that after thyroidectomy a case of acromegalic diabetes uncontrollable by diet or insulin responded well to insulin and showed no glycosuria or hyperglycemia.

Case 15 (27726) gave a history that her mother had had diabetes. At the age of 49 her feet grew from size 7 to 8½, nose and thyroid became enlarged and she gained 36 kg. in 6 months. X-ray of the pituitary fossa showed it to be normal but the fingers showed definite marked tufting. One year later she began to have marked polyuria, polydipsia and polyphagia accompanied by a severe pruritus vulvae. The B.M.R. was +21%. Fasting blood sugars ranged between 200 and 220 mg.% with marked glycosuria. We were unable to follow up her diabetes.

Case 16 (951) died of carcinomatosis after having had acromegaly for 22 years and diabetes for 7 years. The diabetes was diagnosed by a sugar tolerance test, (130, 250, 190 and 130 mg.% with 0.45% sugar in the urine) and he continued to show a mild glycosuria and hyperglycemia in the next 5 years. No dietary or insulin regulation was apparently necessary.

Case 17 (32347) died in March, 1931 after influenza which was followed by convulsions and coma (diabetic origin?). Blood sugars were usually about 190 mg.% with the urine sugar averaging about 0.5%. While in the hospital an attempt was made to measure the sensitivity of the patient to insulin and obstetrical pituitrin. With an initial blood sugar of 380 mg.%, 20 U of insulin were injected. The blood sugar fell in 2 hours to 270, in 4 hours to 250, and in 6 hours to 230 mg.%. When the test was repeated with 20 U of insulin and 1 cc. of obstetrical pituitrin the blood sugar fell from 330 to 260 mg.% in 6 hours. A few days later 20 U of insulin was given when the blood sugar was 260 and in 4 hours it had fallen to 230 mg.%. One would assume that the patient reacted as do most diabetics to insulin, though perhaps a greater drop should be expected.

Case 18 (15505) at the time of her death had "vascular complications, pronounced glycosuria and diabetes." Her B.M.R. ranged from +16% to +85%. At the time when her diabetes was discovered it was +36%, fasting blood sugar was 170 mg.% and urine sugar 1.2%. Insulin with an unrestricted diet had little or no effect. The blood sugars, even on a diet of C 80, P 80, F 120, ranged between 230 and 280 mg.% with a glycosuria varying between 0.5% and "3 plus." The hyperthyroidism was resistant to treatment with Lugol's solution. One cannot say whether it was the secondary hyperthyroidism or the primary hyperpituitarism that was responsible for the insulin insensitivity.

Case 19 (1140) had both acromegaly and an endothelial meningioma of which he died in 1918. The diabetes responded well to dietary management, the patient becoming almost sugar free in 6 days on a "diabetic diet plus 50 grams of bread."

Case 20 (19966) was remarkable because of his abnormally large caloric intake (3500 to 4500 calories per day) with only a moderate hyperglycemia (210 to 327 mg.%) and marked glycosuria (2.5 to 6.3%) without acetone or diacetic acid. The B.M.R. ranged from +13% to +58%. In 1923, insulin was given in doses up to 180 U daily without apparent effect on the blood sugar or glycosuria. In 1929, after having had 32 treatments of radiotherapy, his diabetes was easily controlled on an 'anti-diabetic' diet and a small amount of insulin, 10 U q.i.d. He apparently did quite well until February, 1937 when following a gangrene of the left foot he developed bronchopneumonia. At autopsy a cystic acidophil adenoma of the pituitary was discovered (See table 7 *Case* X). Coronary infarction and a dissecting aneurysm of the lower abdominal aorta were the primary causes of death. The response to insulin therapy in this case roughly paralleled the activity of the hyperpituitarism. At autopsy

microscopic examination of the section from the pancreas showed definite reduction in the number of islands of Langerhans.

Case 21 (36517) developed diabetes after having had acromegaly for 5 years. For 6 months prior to her admission to the Peter Bent Brigham Hospital in May, 1930 her blood sugar values ranged about 200 mg.%. In October, 1929 she began to take 'enormous' amounts of insulin, up to 40 U a day, on which her urine was sugar free. On admission urine gave a red coloration with Benedict's, showed 4+ acetone and the fasting blood sugar was 310 mg.%. Two glucose tolerance tests were made at this time with the following results: fasting, 300; ½ hour after 100 gm. glucose, 380; 1 hour after 100 gm. glucose, 530; 2 hours after 100 gm. glucose, 580; and at the second test were 240, 340, 440 and 510 mg. Apparently the diabetes responded well to insulin and dietary management despite the evidence of a markedly decreased glucose tolerance.

Case 22 (4215) gave no clear-cut history of the onset of acromegaly except for an increase in the size of his hands and feet one year before the onset of glycosuria and the diabetic symptomatology. On a limited house diet his 24-hour urine sugar varied between 4.5 and 5.0% with no diacetic acid or acetone. The B.M.R. varied from +9% to +30%. He died of a hypernephroma, and an examination disclosed a ruptured pituitary cyst.

Case 23 (20989) developed diabetes after having had acromegaly for 3 years. He died 27 days following a transphenoidal operation. He was subject to uncinate attacks, events which are of considerable importance since during an intravenous glucose tolerance test his blood sugar fell to 50 mg.%, and it is possible that these uncinate attacks were due to hypoglycemic periods. The glycosuria responded well to dietary management. His B.M.R. ranged from +12% to +31%.

Case 24 (22075) had the onset of diabetes 5 years after her primary acromegalic changes. In 1929, on admittance to a hospital for treatment of 'styes,' her blood sugar was found to be 210 mg.% and she had marked glycosuria. She was discharged with her diabetes apparently well controlled on a diet of C 70, P 60, F 160 with insulin 10 U before breakfast and supper each day.

Case 25 (41058) developed diabetes after 9 years of acromegaly. Transphenoidal operation for acidophilic adenoma was carried out in May 1932. Diabetes followed in the spring of 1933.

Case 26 (36745) developed headache, coarse features but no prognathism. After 15 years, Dr. W. S. Collens reported progression in the bony enlargement and diabetes which soon required insulin.

Table 1 summarizes the collected data in each case. It would seem apparent that the severity of 'acromegalic' diabetes varies considerably from cases showing a markedly decreased sugar tolerance to those showing only the mildest derangement of carbohydrate metabolism. That the diabetes occurring in certain of these acromegalics displayed the same cyclic intensity as did the acromegaly itself seems clear. In this respect 'acromegalic' diabetes resembles the diabetes associated with hyperthyroidism or infection. The one outstanding feature would seem to be the constantly decreased sugar tolerance manifested by these patients as evidenced by the usual finding of high blood sugars even though the urine sugars were low or normal. The influence of obesity may be seen in table 3 where the weights of acromegalics observed at various periods during the acromegaly are compared with standard weights. Only 8 had been underweight, whereas 73% of the series had been from 10 to 70% overweight.

ACROMEGALY IN A DIABETIC CLINIC

A sharp contrast is evident between the foregoing experience with the development of diabetes in cases of acromegaly and the incidence of acromegaly or hyperpituitarism among diabetics at the George F. Baker Clinic. Among 17,000 cases referred for glycosuria or diabetes since 1898, Joslin has had only 5 cases of acromegaly,

one of which is considered a case of fugitive acromegaly and two of which were referred for special diabetic study after a previous diagnosis of acromegaly. Only 3 cases of excessive height justifying the term 'giantism' were encountered.

Acromegaly in its full-blown state is a rare disorder but easily recognized. Davidoff (31) gave the incidence at the Peter Bent Brigham Hospital as being only 2 out of every 1000 admittances, during the period when Cushing was chief of the surgical staff!

TABLE 3. WEIGHTS OF ACROMEGALIC PATIENTS IN RELATION TO STANDARD WEIGHTS

Age (years)	No. of cases	Below standard weight Per cent			Normal average zone	Above standard weights Per cent							Percentage of each decade below zone
		21–30	11–20	6–10	Per cent −5 to +5	6–10	11–20	21–30	31–40	41–50	51–60	61–70 71+	
11–20	3					1		1			1		
21–30	40			1	5	2	13	8	8	2	1		2.5
31–40	37			1	10	1	7	7	3	3	3	2	2.7
41–50	22		1	2	6	1	7	4	1				27.0
51–60	9			2	2	1	3	1					22.0
61–70	2	1					1						50.0
71+													
Totals	113	1	1	6	23	6	31	21	12	5	5	2	

In Joslin's series the incidence of acromegaly (hyperacidophilism) is about 1 in every 3000 cases of diabetes. This low incidence is in part due to the fact that Dr. Cushing's clinic at the neighboring Peter Bent Brigham Hospital attracted most of the acromegalic patients referred by physicians in New England. Harris (32) mentions only 3 cases of gigantism and 2 cases of acromegaly in his large experience with diabetics. Dr. Russell Wilder of the Mayo Clinic permits us to quote the following statement.

> If we exclude these cases in which the diagnosis of diabetes was questionable and those in which glycosuria was so mild that it readily could be explained by elevation of the basal metabolic rate, it leaves among 218 cases of acromegaly only 12 cases of diabetes representing an incidence of diabetes in acromegaly of only 5.5 per cent. This is not impressive. It is perfectly true that the acromegaly in many of these 218 cases was burned out before the patient came to the clinic, and that glycosuria might have been found in more of them had the patient been examined in the acute stage of acromegaly. It is questionable, however, whether such impermanent glycosuria deserves to be called diabetes mellitus. Furthermore, in some of the cases the diabetes apparently antedated the acromegaly.
>
> My feeling is that too much emphasis has been placed on the occurrence of diabetes with acromegaly, in an effort to correlate clinical findings with the recent discoveries of the physiologists regarding the rôle of the pituitary in carbohydrate metabolism. The incidence of diabetes in acromegaly of 5.5 per cent is almost the same as the incidence of diabetes in 750 cases of adenomatous goiter with hyperthyroidism studied here in the 3 years 1935, 1936 and 1937. That incidence was 5.6 per cent. In more than half of the cases of acromegaly showing diabetes the thyroid contained adenomas, and in these instances the adenoma rather than the pituitary might have provoked the diabetes.
>
> Looked at the other way, namely, considering the incidence of acromegaly in diabetes, the figures are as follows. For the period studied by Yater, (22) 1910 to 1927 inclusive, there were 4,160 new cases of diabetes. This means individual patients. For that period, therefore, if we assume that all 6 cases reported by Yater were cases of acromegaly, the incidence was 0.14 per cent. In the period since January 1, 1928, up to and including all of 1937, there were 5,217 new cases of diabetes. If we accept the diagnosis of acromegaly in all of the 14 cases in which it was made, either positively or with a question mark, the incidence for this period was 0.26 per cent. Combining all cases since 1910 we have a total of 9,377 cases of diabetes and 20 cases of diabetes with acromegaly, or an incidence of 0.21 per cent. However, in 3 of these cases of acromegaly the diagnosis was questionable, and if we accept only 17 cases the incidence of acromegaly is slightly less than 0.2 per cent, which represents about 1 case of acromegaly in 500 cases of diabetes.

The disparity between these incidences does not preclude a pituitary influence in the etiogenesis of every case of diabetes mellitus but is certainly evidence which is strongly against any supposition that hyperpituitarism is the primary cause of diabetes mellitus. One may argue that there need not be the extreme of hyperpituitar-

ism (acromegaly or giantism) present in every case of diabetes to prove a primary pituitary factor. On the other hand, it might be asked why all acromegalics do not develop diabetes, since acromegaly represents the acme of hyperpituitarism.

As Carlson (33) emphasized there is a need for careful clinical studies because of the lack of clinical results in contrast to the amount of laboratory data available. The real obstacle in such studies lies in the fact that there are very few pathognomonic signs or symptoms of any endocrine disorder. Minor degrees of hyperpituitarism are difficult to detect. It is only in the advanced cases and possibly the fugitive cases of acromegaly that the findings including x-ray are usually definite. But if all the cases of diabetes are primarily pituitary in origin or even present secondary hyperpituitarism, it is perfectly evident that the hyperpituitary state involved is one without such obvious stigmata as are found in acromegaly or giantism.

DIAGNOSTIC PROCEDURE

In the search for hyperpituitary states, we have relied upon the clinical history, evaluation of the symptomatology and physical findings, with special laboratory tests including x-ray studies of the skull and hands. Few laboratory findings warrant even the supposition of a hyperpituitary state. An increase in the blood uric acid and eosinophile count are only suggestive, as was brought out by Rowe (34). Below is given in abbreviated form a suggested examination for the detection of hyperpituitary states.

EXAMINATION FORM FOR DETECTION OF PITUITARY DISEASE

HISTORY—Complete clinical history with special emphasis on:

1. *Familial*—evidence of overweight, overheight, coarse features, race, sterility, fertility, blindness, brain tumors, diabetes, thyroid disease, other endocrine disorders.
2. *Personal*—age, sex, race, marital history, onset of puberty, infections of paranasal sinuses, tonsillitis, chronic fevers or infections, sudden increase in weight or height, thyroid disturbances, menstrual disturbances, headaches, visual disturbances.

SYMPTOMS

Disturbance of menstrual cycle	Polyphagia
Headaches	Polydipsia
Visual disturbances	Constipation
Excessive perspiration	Rhinorrhea
Drowsiness and lethargy	Photophobia
Diminished libido sexualis	Uncinate attacks
Asthenia	Failing memory
Paraesthesias	Epistaxis

PHYSICAL FINDINGS—Complete physical examination including opthalmoscopic and neurologic examinations with special emphasis on:

1. Physical measurements—Height, weight, sitting height index, span, size of hands, size of feet.
2. Enlargement of jaw.
3. Malocclusion of teeth.
4. Prominent paranasal sinuses.
5. Character of skin—texture, wrinkling, hypo- or hypertrichosis, excessive perspiration, fibromata mollusca, hyperpigmentation.
6. Examination of other endocrines—thyroid, ovaries.
7. Kyphosis.
8. Enlargement of viscera (heart, liver, kidneys, spleen).

LABORATORY TESTS

Urine: Day-night ratio, total volume, specific gravity (2-hour renal test), sugar, diacetic acid and acetone, nitrogen, phenolsulphonphthalein.

Blood: R.B.C., HB., W.B.C., differential, serology, sugar, N.P.N., cholesterol, uric acid, Ca, P, chlorides, CO_2 combining power, creatinine, iodine, estrin, prolan, glucose tolerance, insulin sensitivity.

X-ray: Skull including sella turcica, jaw and hands.

Metabolic Studies: Basal metabolic rates, respiratory quotients.

CASE REPORTS

Three new cases of acromegaly in the experience of the George F. Baker Clinic, together with certain patients whose examinations showed suspicious but not conclusive evidence of hyperpituitarism are summarized. Two other cases of acromegaly (5447 and 4219) have been discussed previously.

Case 16715 a male, was 60 years of age, married and a dentist. His height was 170.2 cm., weight 73 kg. on March 28, 1938. The father had diabetes, and died of a cerebral accident at 78. The mother died of pneumonia at 66. There was only one other child, a brother who is living and well at 59. All members of his family were said to be coarse-featured.

The patient had measles, mumps, whooping cough and frequent attacks of tonsillitis as a child. He was married at 25; his wife and the only child, a daughter, aged 32, are well. His weight had increased gradually from 61 kg. at the age of 25 to 100 kg. at 52. His wife stated, however, that he had gained in weight very rapidly just after marriage. Diabetes began indefinitely, probably about July of 1931, at which time he noticed polyuria, polydipsia and a loss of strength. Glycosuria was discovered in July, 1932. Insulin was begun in 1936, and at the time of the examination the dose was 20 U of regular insulin each morning. The patient was uncooperative during the direct questioning, admitted no symptoms other than the strictly diabetic ones since 1931. He was well developed and well nourished, heavily bearded and coarse-featured. The jaw appeared large and there was definite prognathism as shown by protrusion of his lower teeth. The skin was coarse, thick and moist, the tongue seemed large and his speech was thick and slow. There was a moderate kyphosis. The hands were large with spade-like fingers. The heart did not appear enlarged on percussion, the lungs were emphysematous, the thyroid normal size, the liver down 3 to 4 cm. in right nipple line. The blood pressure averaged 120/80. There was a small necrotic area between the 4th and 5th toes of the right foot, this being his entering complaint. Pulsations in the dorsalis pedis arteries were absent. Neurologic and ophthalmoscopic findings were non-contributory. The urine volume was low, averaging between 700 and 1500 cc. daily, with the night volume almost equalling the day volume. The specific gravity ranged between 1.014 and 1.024. Sugar ranged from only a slight trace to 1% for the 24-hour amounts.

The red blood count was 4,400,000, the W.B.C. 16,400, hemoglobin 83%; differential P/76, L/16, LM/6, EO/1, Bas/1. Result of the Hinton test was negative. The N.P.N. varied between 26 and 30 mg.%. Cholesterol on one day was 112, uric acid 2.6 mg.%.

A test for insulin sensitivity by the Himsworth method was carried out using 53.7 gm. glucose. First the glucose (53.7 gm.) was given without insulin in the fasting state and the following blood sugar values obtained; fasting 140 mg., ½ hour 145 mg., 1 hour 160 mg., and 1½ hours 214 mg.%. This abnormal curve we attributed to delayed absorption of the glucose solution. When the test was repeated, but with the injection of 9 U of insulin, the blood sugar values were as follows: fasting 176, ½ hour after glucose and insulin, 164, 1 hour after, 204, 2 hours after 196, and 3 hours after, 220 mg.%. Such a curve would indicate relative insensitivity to insulin. Below is given a sample 5-day period to show blood sugar values, corresponding 24-hour urine sugar amounts, diet and insulin.

Day	*Blood Sugar mg. %*	*Urine sugar*	*Diet*	*Insulin*
3/31/38	230 fasting	trace	C 118, P 73, F 93	12+40
4/1/38	220 fasting	O	C 120, P O, F O	40
4/2/38	210 cap. blood 10:30 A.M.	trace	C 112, P 25, F 56	12+40
4/3/38	——	trace	C 110, P 16, F 21	16+40
4/4/38	250 fasting			
	190 cap. blood 11 A.M.	trace	C 75, P 18, F 42	20–4–20

This period represented the 4 days immediately following amputation of his right leg. Blood iodine was 6.5 γ% (normal). The result of a test for blood prolan was negative. Results of urine studies for estrin and prolan content on the same date were negative. X-ray disclosed a sella turcica measuring 1.7 by 2.0 cm. an enlarged jaw, tufting of terminal phalanges of

both hands—"findings consistent with acromegaly." The B.M.R. was +12%. The patient was discharged sugar free, taking 6 U of regular insulin and 24 U of protamine zinc insulin on a diet of C 150, P 85 and F 105.

The characteristic physical findings and the indisputable x-ray evidence of an enlarged sella turcica with enlargement of the jaw and paranasal sinuses and tufting of the terminal phalanges of both hands established the diagnosis of acromegaly. Hyperglycemia with only minimal glycosuria is noteworthy but not rare among

TABLE 4. INSULIN DOSAGE, REACTIONS, AND LABORATORY DATA. *Case 16716*

Date 1938 March	Daily insulin		Reactions hypoglycemia	Blood		Urine sugar	Diet		
	Reg. I[1]	PZI[2]		Sugar	N.P.N.		C	P	F
	U	U		mg.	mg.	gm.	gm.	gm.	gm.
12	16	52		120	30	21	149	80	98
13	20	52	+	30		35	149	80	98
14	14	48	++	200		56	151	62	101
15	22	44	+	230		33	150	61	100
16	32					35	150	61	100
17	32			290	32	84	112	94	92
18	28					91	150	61	100
19	46					74	122	49	72
20	44		+			45	150	61	100
21	60		+	180		53	150	61	100
22	10	44				133	150	61	100
23	10	44				85	151	62	111
24	18	48		60		6	151	62	111
25	14	48	++	50		3	140	73	124
26	8	44	+	50		0	140	73	124
27	8	40		50		44	140	73	124
28	6	38		100		0	140	73	124
29	10	38		70		12	140	73	124
30	4	36		130		35	152	81	126
31	4	38		70		2	152	81	124
April									
1	4	38				66	152	81	124
2	4	38	+	100		2	152	81	124
1939									
April 22	4	30		170		54	184	84	122

[1] Reg. I = unmodified insulin.
[2] PZI = protamine zinc insulin.

ordinary diabetics of his age with arteriosclerotic complications. Apparently, however, the hyperpituitarism has either burned out or is dormant.

Case 16716, a white male sheet-metal worker, 167.7 cm. in height, 67 kg. in weight, age 50, was first seen March 11, 1938. Sitting height was 88.9 cm., span 174.5 cm. The father died at 60 of an unknown cause, mother at 72 of a cardiac condition. He has 2 brothers, 51 and 46 years old and 3 sisters 55, 48 and 45, all living and well. The family were said to have been all heavy-featured but none overheight or overweight.

The patient was married at 25, had 4 children now 10, 18, 21 and 24 years old. He had measles, mumps and pertussis as a child. Between the ages of 25 and 30, he gained from 60 kg. to 71 kg. Five years ago he noticed that he needed larger gloves. Glycosuria was first discovered in September of 1934 and polyuria, polydipsia and loss of weight had been prominent only since January 1934. His entering complaints were nocturia, nervousness, failing vision and difficulty in controlling glycosuria. Insulin had been begun in 1934 at which time he was taking 20 U of regular insulin before breakfast and supper. On admission he was taking 40 U of protamine zinc insulin before breakfast and 30 U of protamine zinc insulin before supper. No insulin reactions were noted but the patient complained of severe headaches daily on awakening.

Examination showed a coarse-featured, heavily jawed, slowly speaking, stockily built white male. Skin was coarse-textured and very moist from increased perspiration. Hands were enlarged with blunted widened finger tips. There was no kyphosis. The tongue seemed slightly enlarged, tonsils were huge. Thyroid was normal in size. The cardiac apex was 8.5 cm. to the left of the mid-sternal line with a distinct systolic murmur loudest in this region. Liver was 2 cm. down in the right mid-clavicular line. There was a moderate palpable arteriosclerosis in the peripheral arteries. Results of a neurological examination were normal. Ophthalmoscopic examination revealed a high degree of myopia in both eyes with some pigment disturbance in the right fundus. Visual fields were normal. Otherwise the findings were not remarkable.

The total daily urine volume varied between 1050 and 2500 cc. with the night volume exceeding the day volume; half the time, the average night amount for 22 days exactly equalled the day amount at 868 cc. The specific gravity varied from 1.015 to 1.027. Nitrogen determinations were made daily. An apparent positive nitrogen retention may have been due to imperfect absorption of protein from the intestinal tract. The urinary creatinine excretion on 3 days was 1.19, 1.32 and 1.38 gm. Table 4 shows the daily insulin dosage, diet, urinary sugar values and blood sugar range.

The result of a Hinton test was negative, R.B.C. was 4,760,000, Hb. 100%, W.B.C. 11,200, differential P/70, L/14, LM/14, E/2. Blood uric acid was 2.9 mg.%. Plasma cholesterol was 212 mg. The test for blood prolan was unsatisfactory. The result of a test for urine prolan was reported as questionably weak positive. Blood iodine was 12.77%. An insulin sensitivity test in which 51.6 gm. of glucose orally and 8.6 U of regular insulin intravenously were given resulted in the following blood sugar values: fasting, 350 mg.%; ½ hour after glucose and insulin, 416 mg.%; 1 hour after glucose and insulin 414 mg.%; 1½ hours after glucose and insulin, 404 mg.%. Such a curve indicates a moderate insensitivity to insulin. However, the fact that he had 9 severe insulin reactions during his stay following slight changes of a few units in his insulin dosage is clear proof that he was really sensitive to insulin.

The x-ray report was as follows: "Skull normal, sinuses are large, jaw increased markedly in size. Hands are large for patient's height. Slight tufting of terminal phalanges. Cortices are heavy." Metabolic studies were carried out at the Carnegie Nutrition Laboratory through the courtesy of Dr. Thorne Carpenter. Respiratory quotients are given here as determined in the Carnegie Laboratory (see table 5). Non-protein respiratory quotients for periods 4 to 6 averaged 0.681; for periods 7 to 9, 0.694. The patient excreted 3.1 gm. sugar in 3 hours and 18 minutes; the time required for the examination. If this urine sugar had been burned, the non-protein respiratory quotients would have been for periods 4 to 6, 0.694, for periods 7 to 9, 0.708. The patient developed slight acidosis in the hours preceding the experiment so that during the experiment, the plasma CO_2 combining power fell to 39 volumes per cent, the urine contained diacetic acid and the blood sugar was 0.29% (290 mg.). During this acidosis the increase in oxygen consumption which was expected to attain a maximum at two to three hours, was not observed. Whether this absence of a specific dynamic action was related to acromegaly or to the acidosis is uncertain. Goldzieher (30) quoting divergent data and opinions, affirms that its absence indicated hypopituitarism. In the presence of acromegaly, it is possible that a tumor may by pressure finally lead to atrophy of the anterior portion of the pituitary.

The high B.M.R., +34% was not due to acidosis since the O_2 consumption was slightly less on March 17 when slight acidosis was present than on March 19 when no acidosis was present. We believe that the high rates on March 17 and March 19 were due to hyperpituitarism and that the steady decline in metabolic rates to +22% on December 11, 1938, and later to +1% on April 23, 1939 was due to subsidence of this wave of hyperpituitarism. If one assumes that the acromegaly had subsided, then the change in the specific dynamic action of protein is unexplained. The elevation of the blood iodine in March, 1938 together with the positive test for blood prolan, indicated hyperpituitarism with secondary hyperthyroidism.

Improvement in diabetic control is clearly seen in Table 4 from March 12 to April 2,

1938. The increase in insulin dose which had been necessary during the fall of 1937 no longer was required. Yet the record of April 22, 1939 when the metabolic rate had returned to normal shows little if any further improvement, as if the basic severity of the diabetes had been scarcely affected by the wave of hyperpituitarism of fully 18 months duration.

Case 14320 was a white housewife, 57 years old at her first visit in January 1936. There was no family history of extreme height or weight or diabetes. She had had 9 children, of which 2 died at birth, 2 more died in infancy and 5 were well at the ages of 29, 27, 24, 21 and 15.

Onset of diabetes occurred between October, 1929 and October, 1930, she lost 17 kg., and glycosuria associated with polyuria, polydipsia and polyphagia was discovered.

In 1925, the hand and ring finger were so large that she could hardly get a ring off. Since that year she has had to have the ring enlarged several times. In 1934 she began to gain

TABLE 5. SPECIFIC DYNAMIC ACTION OF PROTEIN IN AN ACROMEGALIC DIABETIC BEFORE AND AFTER CONTROL OF DIABETES DURING SUBSIDENCE OF HYPERPITUITARISM

Date	Period time—hours	R.Q.	Total O_2 per minute	H & B[1] per cent
March 17, 1938	1	0.700	286	+34
	2 fasting	0.746	279	
	3	0.770	292	
	(200 gm. beefsteak were given here)			
	4	0.690	334	
	5 1	0.710	322	
	6	0.720	309	
	7 2	0.695	308	
	8	0.730	297	
	9 3	0.730	295	
March 19, 1938	10	0.700	295	
	11 fasting	0.703	294	+35
	12	0.712	293	
Dec. 11, 1938	13 fasting		242	+22
	(200 gm. beefsteak were given here)			
	14 ½ hour		265	
	15 1 hour		265	
	16 2 hours		279	
	17 3 hours		295	
April 22, 1939	18 fasting (3 periods)	0.720	228	+10
April 23, 1939	19 fasting (5 periods)	0.780	207	+1

[1] H and B=Harris and Benedict standards.

weight "for no apparent reason" and on her first visit weighed 71.7 kg. and was 168 cm. tall. Her maximum weight was 72.6 kg. in 1927; menopause occurred at the age of 50.

Blood sugar values in January, 1936 were 150, 330 and 520 mg.%, the latter associated with 6.4% sugar in the urine. She became sugar-free on a diet of C 100, P 60, F 50, with regular insulin, 15 U before breakfast and supper each day.

The x-ray report was as follows. "The pituitary fossa is much larger than normal. There is no erosion of the wall of the sphenoid sinus or clinoid processes. The enlargement of the pituitary fossa is probably due to a physiological hypertrophy of the pituitary gland. The bones of the fingers are broad suggesting acromegalic deformity."

Physical findings included typical acromegalic facies, large hands with broad square-ended fingers, palpable marked arteriosclerosis, an enlarged heart and liver and a macular eruption on the arms and legs. Blood pressure was 134 systolic and 80 diastolic. Breasts were noted as being smaller than normal, tongue and tonsils as being enlarged. Results of neurological and ophthalmoscopic examinations were normal. The patient refused hospitalization for further study.

The acromegaly was of at least 11 years duration with diabetes mellitus inter-

vening after 4 years. Diabetes was moderately severe (signs of acidosis) but was apparently amenable to dietary regulation and small doses of insulin.

During the past year in 1500 diabetic admissions in the George F. Baker Clinic we have found 2 cases of acromegaly (*16715* and *16716*). Three cases (5520, *11072* and 16090), males, attained unusual height at puberty and developed diabetes at 27 years, 14 years and 19 years respectively. In a group of 3 women, striking obesity, hirsutism and amenorrhea were associated, suggesting the diabetes of bearded women described by Shepardson and Shapiro (42), but usually associated with adrenal cortical tumor. One case (*11129*) had such a tumor at autopsy.

Table 6 shows a comparison of the incidence of various signs and symptoms in 100 diabetics with 100 acromegalics. The diabetics were routinely studied for symp-

TABLE 6

Symptoms, signs of acromegaly as recorded in clinical histories	100 Cases of acromegaly	100 Cases of diabetes	Comment
Enlargement of acral parts	100	6	
Enlargement of sella turcica (x-ray)	93	0	8 examined
Disturbances of menstrual cycle	87	30	50 females studied
Headaches	87	35	
Complete amenorrhea	73	10	At least 1 yr. duration
Increased B.M.R.	70	17	47 examined
Visual disturbances	62	27	
Excessive perspiration	60	25	
Hypertrichosis	53	9	
Cutaneous pigmentation	46	3	
Drowsiness and lethargy	42	1	
Gain in weight	39	51	Sudden increase
Diminished libido sexualis	38	9	
Asthenia	33	26	
Low B.P. (less than 120)	30	26	
Paresthesias	30	24	
Fibromata molusca of skin	27	4	
Polydipsia	25	81	
Enlarged thyroid gland	25	8	
Glycosuria	25	100	
Constipation	20	43	
Vomiting	16	7	
Rhinorrhea	15	0	
Photophobia	12	0	
Uncinate attacks	7	0	
Failing memory	7	1	
Decrease of body hair	7	0	
Persistent lactation	4	1	
Failure of breasts to develop	4	5	
Epistaxis	3	0	
Choked discs	3	0	

tomatic evidence of a disordered relationship between the various endocrine glands without regard to their presenting complaints or outstanding features in their histories or general examinations. We are fully cognizant that such a study lacks scientific accuracy, the results being dependent on the observer's interpretation of the symptoms and signs. It is possible that a series of another hundred cases might show widely varying results. Certainly objective findings, such as basal metabolic rates exceeding 10% and blood pressure determinations, may be stressed. We do not hold the view that the somewhat similar incidence of certain symptoms in the diabetic series represents evidence of a primary pituitary influence in diabetes mellitus.

Until more accurate diagnostic procedures are known, we can only entertain suspicion as to the actual involvement of the pituitary in these cases. A reciprocal relation between the anterior pituitary and pancreas may explain the fact that in

diabetic patients with such symptoms as amenorrhea, impotence, and a low basal metabolism, these symptoms follow the diabetes rather than precede it. It is reasonable to believe that the disturbances in the hair, skin and gonads observed in diabetics are secondary to the diabetes, and perhaps due to impaired pituitary function dependent upon the pancreatic failure.

Weights of visceral organs in acromegaly and diabetes. Davidoff divides the clinical course of acromegaly into 3 periods: (*a*) Early period, general over-growth, gain in weight, menstrual disturbances, due to overactivity of the pars anterior of the pituitary glands; (*b*) Middle period, neighborhood symptoms from pressure, visual disturbances, increase in sella turcica, tumor signs and (*c*) Final period, polyphagia, polydipsia, etc., due to visceral splanchnomegaly and secondary effects of the other endocrine organs. Davidoff and Cushing emphasize that acromegaly results in an overgrowth of all tissues. The splanchnomegaly apparently is not due to the laying down of excess fat or other abnormal deposits but is an hypertrophy or more likely, an hyperplasia of all the tissue elements. It is the general splanchnomegaly which is characteristic of the disease, the difference between gigantism and acromegaly being purely a matter of chance with regard to the time of development of the disease. The splanchnomegaly is shown by the increased weight and size of the lungs, heart, kidneys, liver and spleen, and especially the tendency to adenomatous enlargement of the thyroid, parathyroid, thymus and adrenal glands, as well as the pancreas. The atrophy of the testes and the cystic changes in the ovaries are described in detail by Cushing. Since many cases of acromegaly developing in adult life may show no increased height but instead actual shrinkage due to the kyphosis, the weights of the organs actually involved become of greater importance in diagnosis. Indeed, this splanchnomegaly is usually disproportionate to the increase in the size of the body.

It is generally assumed that the enlargement of the acra, as well as the splanchnomegaly in acromegaly is due to the excessive secretion of some substance or hormone secreted by acidophilic cells of a pituitary adenoma of the anterior portion. One may suppose that the excessive development of diabetes in such patients is also due to this same agent acting either directly or indirectly upon the islands of Langerhans. In either case, under the influence of such a substance or hormone, we would expect to find some such relationship, between the weight of the pancreas and the weight of other organs in diabetic patients, as occurs in acromegaly. To test this hypothesis, we therefore have tabulated the weights of tissues in 11 acromegalic patients in table 7 together with the weights of tissues in groups of diabetic patients and in cases of Simmonds' disease. Four of the acromegalic cases (No. I, II, VII and IX) were reported by Cushing and Davidoff (43).

It will be seen that the acromegalic patients are divided into three groups: (*a*) those without diabetes, (*b*) those in whom the acromegaly was fugitive in the sense that the over-growth in the acra and the jaw developed at ages varying from 17 to 38 years, but no further changes followed, (*c*) those with diabetes and acromegaly. Definite acromegaly was present both in the patients with acromegaly alone and in those with diabetes in addition to acromegaly. It is true that the average weight of the heart was 605 gm. in patients with diabetes in contrast with 743 gm. in acromegalics without diabetes. The liver of the diabetics on the average weighed 2645 gm., whereas the liver of the acromegalics without diabetes weighed 2937 gm. The kidneys of the diabetics weighed 671 gm., whereas the kidneys in acromegalics without diabetes weighed 633 gm. Similar differences appeared in the weights of the spleen and adrenals. Unfortunately, there is no record of the weight of the thyroid gland in the acromegalic with diabetes, although in two instances the thyroid was

noted as being large. It is of interest that the pancreas in both these groups weighed on the average 149 gm.

The group classified as fugitive acromegalics requires some further explanation. In each autopsy a mixed tumor of the pituitary was found. The tumors contained both chromophobe and chromophilic tumors. *Case 4* died of a subdural hemorrhage. *Case 5* died of myocarditis and chronic interstitial nephritis, and thus an adequate explanation for the enlargement of the heart was found. *Case 6* died of a hemorrhage into the

TABLE 7. COMPARATIVE WEIGHTS OF ORGANS IN DIABETES AND PITUITARY DISEASE

	Autopsies	Ht.	Wt.	Heart	Liver	Kidneys	Spleen	Thyroid	Adrenals	Pancreas
		cm.	kg.							
	Normal Male	172	70	350	1500	300	200	25	12	80
Acromegalics without diabetes	I. Male, age 52 Duration 30 years[1]	185	97	1050	3150	853	535	310	43	225
	II. Male, age 40 Duration 13 years[1]	198	122.4	480	3380	565	385	105	43	83
	III. Female, age 39 Duration 26 years	164.5	62.1	700	2280	480	260	20	33	140
	Average	183	94	743	2937	633	393	145	39	149
Fugitive acromegalics	IV. Male, age 63 Duration 25 years	177	66.2	280	1545	200	200			
	V. Female, age 55 Duration 23 years			500	1700	350	205	140		
	VI. Male, age 57 Duration 40 years	182	70.6	300	1720	300	220			
	VII. Male, age 42 Duration 13 years	172	98.6	330	2015	360	450			
	Average	177	78	352	1745	303	269			
Acromegalics with diabetes	VIII. Male, age 35[1] Duration 15 years	173	100	1000	2480	650		100	30	150
	IX. Female, age 51[1] Duration 21 years	157	68	460	2500	695	240		18	
	X. Male, age 58 Duration 23 years	168	91	600	2900	460	330	'enlarged'	28	180
	XI. Male, age 42 Duration 17 years	187	108	360	2700	880	240	'enlarged'		117
	Average	171	92	605	2645	671	270		25	149
Diabetics Large pancreas	Group I (14 cases) more than 120 gm.	168	88	410	1938	382	269	24	'normal'	152[2]
	Group II (23 cases) between 100–120 gm.	165	88	380	1645[2]	385	150	21	'normal'	106
Small pancreas	Group III (13 cases) 40 gm. or less	164	78	359	1630	312	115	19	'normal'	32
Simmonds' disease				175	982	237	85	20	19	67

[1] See Cushing, Harvey and Davidoff, L. M., Monograph 22, Rockefeller Institute for Medical Research, for complete discussion of these autopsies (our reference 43).

[2] Excluding one case with carcinomatous masses.

pituitary cyst or a cystic area in the pituitary tumor. *Case 7* died of congestion of the lungs, probably cardiac. Possibly the excessive function of the acidophilic cells of the tumor has been checked after a period by some influence such as the pressure of the chromophobe portion of the tumor.

In all the acromegalic patients, with and without diabetes, acidophilic tumors were found. The size of the tumors varied from a tumor so small that as in *Case 1*, who suffered to the fullest extent from the classical picture of acromegaly, it had produced no enlargement of the sella turcica in a patient, nevertheless, to tumors which had extended far beyond the confines of the sella turcica as in *Case 2*.

The diabetic patients are divided into three groups, according to pancreas weights. In the 14 cases in which the pancreas weighed more than 120 gm. is excluded one

case with a large carcinoma of the pancreas, weighing 450 gm. In the others fatty invasion largely accounted for the excessive weight. In the diabetic group with small pancreases weighing 40 gm. or less is included one pancreas weighing only 15 gm., of a woman who died in the Deaconess Hospital after observation for nearly 10 years. There was no very close correlation between the weight of the diabetic patient and the weight of the pancreas. The average maximum weight of the diabetic patients with large pancreases was 88 kg. and average height 166 cm. The average weight of the diabetic patients with small pancreases was 78 kg. and the average height 164 cm. The striking features in the diabetic group are, therefore, the lack of correlation between the weight of the pancreas and the weight of the heart, liver, kidneys, spleen or thyroid. As a matter of fact, in the first group of diabetic patients with large pancreases the average weight of the liver, 1978 gm., is due to the inclusion in this group of 6 patients who had a definite reason for congestion and enlargement of the liver, including chronic cardiac failure and cirrhosis of the liver. In contrast two cases of Simmonds' disease showed great reduction in weight of all organs. There is no suggestion of any influence upon the weight of tissues of the diabetic patient by any substance such as is responsible for the enlargement of the skeleton and the visceral organs in patients with acromegaly.

DISCUSSION

The outstanding fact is the great rarity of acromegaly or gigantism in the diabetic clinic. White (45) finds that a considerable portion of diabetic children are above the average height for their age at onset of diabetes and also show earlier bone and dental development with earlier manifestations of secondary sex characters. So long as such phenomena as the increasing height of university freshman and of recruits in the Japanese army occur under the influence of improvement in hygiene and nutrition, it must be admitted that the final standards of normal growth are not settled. True cases of gigantism with height exceeding 6 ft., 2 in. and enlarged bones generally, occurred in 3 cases and true acromegaly in 5, a total of 8 instances among 17,000 patients with glycosuria or diabetes. On the other hand, Dublin and Marks (35) found no evidence that excessive height by itself indicates pituitary abnormality, and found only 5 deaths from diabetes among the 21,409 subjects studied. Moreover, all of these deaths from diabetes "occurred among older men, four of whom were definitely overweight and every one of the deaths was recorded in the 6 feet, 2 inch group, the shortest height considered in this study."

Acromegaly in every case preceded the diabetes by an average period of 9.2 years in this series. In the 29 cases reported here, females numbered 19 and males numbered 10, a distribution comparable with the distribution for diabetes generally. Acromegaly begins in the late teens or at least before the age of 35 and in this series of 29 cases the acromegaly began before the age of 30 in 16 cases. The best evidence of the importance of the diabetes and its serious character is obtained from the statement of Cushing (43) that among the 44 acromegalic patients studied at autopsy whom he listed for reference, 11 (or 25%) died of diabetic coma. These cases occurred in the preinsulin period. It is by no means certain that the same agent which produced the skeletal and visceral changes of the acromegaly also caused the diabetes. The diabetes, once established, was not different from diabetes as seen in the diabetic clinic. The nature of the substance undoubtedly elaborated in large amount by the acidophilic tumor of the pituitary is to be considered in relation to its extraordinary effect upon the size and weight not only of the skeleton but of the internal tissues and particularly the adenomatous changes produced in certain of the other endocrine glands.

Gigantism or hyperplasia of tissue may be a vastly complicated mechanism involving the interaction of many influences, constitutional, nutritional and endocrine.

Patients with acromegaly have general enlargement of tissues both in the presence and in the absence of diabetes. Diabetic patients without signs or evidence of acromegaly do not show any such evidence of splanchnomegaly. However, there is one point at which the possibility of pituitary activity and of the influence of diabetes meet, namely, the enlargement of the liver. Enlargement of the liver does occur in diabetic patients under poor control. There is also a factor in the pituitary which causes deposition of fat and one which causes retention of glycogen (glycostatic). In animals made diabetic by the removal of the pancreas it is possible to produce a wide range of change in the proportions of glycogen and of fat in the liver by dietary changes and by the use of insulin.

Effect of pituitary extract on the chemistry of the liver. Marks and Young (36) have studied the effect of various glandular extracts upon the chemistry of the liver. Young found that the pituitary factor which caused insensitivity to the hypoglycemic action of the injection was present in the prolactin fraction as it is ordinarily prepared but it could be distinguished from the gonadotropic and thyrotropic hormones and from prolactin. He called it 'glycotropic factor.' They injected various types of extracts into young rabbits. They found in the first place that such rabbits, when previously treated with an anterior pituitary extract, did not show an increase in liver glycogen when insulin was injected. However, it was true that glycogen had already been increased by the previous injections. A crude extract of the anterior pituitary as well as prolactin and the thyrotropic hormone, did increase the weight of the liver. However, they showed that the increase in the weight of the liver was not due to a true hepatomegaly but to the presence of extra fat, water and glycogen. It seems likely, therefore, that although considerable changes in the liver size and weight can be produced by variations in the activities of the anterior pituitary and that, therefore, one might postulate the possibility that in diabetes some of the liver enlargement could be explained by variations in the activity of the anterior pituitary, nevertheless, in true acromegaly we deal with a different situation in which an actual hyperplasia of the liver has taken place as is true with other organs. This is particularly well shown by Lee (37) who points out that not all body tissues and organs participate equally in their response to anterior pituitary growth extract. Young organs respond especially well. The size of the liver depends somewhat upon the state of nutrition of the animal when treated with the anterior pituitary growth hormone. Indeed, its response can be used as a method of assay for the growth hormone. Lee points out that treatment with the growth hormone does not alter the normal architecture of the liver. The increased size is due to true hyperplasia of all the liver components, in the same proportions that existed initially. It is true for animals whose pituitary glands have been removed and who are then treated with the growth hormone. One feature of this treatment is that during the administration of the growth hormone there is a decrease in urinary nitrogen which may be very considerable. In *Case 16716* a low value for urinary nitrogen during the period of his diabetic treatment may be taken as evidence that his acromegaly was not entirely quiescent. Lee showed that repeated assays of fresh anterior lobes from calves, steers and old beeves show no difference in the growth hormone content with increasing age. The content is about the same in 100 gm. of gland, and therefore, is greater in absolute amounts in older animals with larger glands than it is in the young. It appears, therefore, that the amount of growth hormone needed is greater in those animals than in young ones and that growth in the young animals is dependent quite as much on

the state of the tissue cells and their responsiveness to growth hormone as it is on the presence of a definitive amount of the hormone itself. Similarly the effect of the increased secretion of the hormone in acromegaly upon the islands of Langerhans may well depend upon their constitutional integrity. The existence of an hereditary predisposition might be the decisive factor. The possibility that the anterior pituitary hormone produces glycosuria and hyperglycosuria by increased glycogenesis from protein in the liver must be regarded as an accessory rather than as a principal agency.

Another striking difference is found if we consider the ovaries and the testes. Cushing described in acromegalic cases at autopsy, frequent changes in the ovaries, consisting of the formation of graafian follicles which become cystic. The cessation of menstruation in acromegaly is permanent. Although the changes in the testes are said by Cushing to be difficult to classify, nevertheless, the fact remains that even if there is an early period of excessive virility and libido, nearly all acromegalic men lose libido and become impotent within a short time. In strong contrast are the changes in diabetes. Although loss of fertility and menstruation in diabetic women, together with impotence in diabetic men, were formerly quite common, it is true that almost without exception the use of insulin will restore sexual vigor and fertility before the age of 45 years.

Why do not all patients with acromegaly get diabetes? The first of Cushing's 4 autopsied cases developed acromegaly in 1892 at the age of 23 years. He became obese, had all the characteristic features of acromegaly, lived for 28 years and died without diabetes. His tolerance of carbohydrate was so high that at one time he was given 100 gm. a day of Armour & Company's tablets of anterior pituitary lobe extract in order to try to produce glycosuria with 150 gm. of levulose. The second case, a farmer, whose rapid growth began at the age of 13, became an acromegalic giant and died at the age of 40 after an operative attempt to remove a large tumor of the pituitary. His weight reached 281 lb., he had an exceptionally high tolerance for glucose and never had glycosuria or an increase in blood sugar. In a series of 153 acromegalics, 47 died within 10 years of onset of acromegaly and, therefore, may be said to have had acromegaly too short a time to develop diabetes; 26 had diabetes for an average duration of 7.5 years. Among these 127 cases, without known diabetes, a family history of diabetes was obtained in 3 cases, 2%, whereas the acromegalics with diabetes had diabetic relatives in 19% of the cases. An important fact is that over-activity of the gland is followed by under-activity. As the tumor grows by simple pressure it may bring about an atrophy of the acidophilic portion of the tumor and so a stage can be reached in which there is relative quiescence. This stage may exist for many years. Indeed, in the first case of this series, Cushing's explanation was that the obesity and the high carbohydrate tolerance were due to a subsidence of the over-activity. However in this case the pituitary was not enlarged at autopsy.

No such characteristic histopathological changes in the pancreas of subjects who have had acromegaly and diabetes were seen as have been produced by Young by the injection of anterior pituitary extract in dogs. Conversely, no one lesion can be found in the pituitaries of diabetics which might indicate any such factor. Warren (37) in a study of the pituitary glands in 18 cases of diabetes and from random sections from 26 additional cases, found no significant change. He also states that any lesion found in the pancreas of diabetic individuals either involving the island tissues, the acinar tissue, or both can be duplicated in the pancreases of non-diabetics. Obviously then the relation of acromegaly (hyperpituitarism) is not to be found easily in organic pathological study.

One may hypothecate a state of affairs in which a normal secretion of the growth

hormone is relatively excessive for the pancreas hereditarily defective in a given individual or a state of affairs in which the pituitary, apparently normal from the cytological examination, nevertheless secretes an altered hormone which was in some way toxic so far as the islands of Langerhans are concerned. The abnormal hypertrophy of organs in acromegaly may result in a functional as well as organic imbalance between the various glands of internal secretion and the increased frequency of diabetes in acromegalics may be due to a functional inadequacy of the pancreas even though the pancreas may be much larger than the pancreases of normals or of diabetics without an associated hyperpituitary disease. Such an argument may be applied to the interpretation of the effect of hyperpituitarism on the other endocrine glands. In this connection, a case described by Lammli (29) is pertinent. The patient suffering from acromegaly and diabetes mellitus and having a metabolic rate of 50% above normal was not benefited by the removal of the eosinophilic adenoma. Later thyroidectomy brought about disappearance of the insulin resistance, glycosuria and hyperglycemia.

Cannon (39) states that the normal blood sugar level of 100 mg. % is due to an adjustment between two antagonistic agencies, the insulin (or vago-insular) and the sympathico-adrenal systems. A disease like acromegaly producing splanchnomegaly though not necessarily the same degree of hyperplasia of the various organs, would seem a most likely predisposing agent in producing a disequilibrium between the pancreas and the other organs. We may be wrong in applying those standards of normal blood sugar to acromegalics. In other words, the homeostasis of the blood sugar may allow for much higher blood sugars than 100 mg. % in acromegalics.

Another possibility exists in that as a result of the damage to the islands of Langerhans a secondary change might occur in the anterior pituitary. It has been shown that thyroidectomy is followed by changes in the function of the pituitary gland. The striking fact is that practically all the delayed growth and dwarfism in diabetic children, which occurs in perhaps one out of twenty, begins after the diabetes. White (40) finds it is not explainable in more than a small portion of the cases as the result of failure to use insulin or of inadequate and careless dietary control. Rather it is true that a careful study of the other varied and important factors in growth must be made in each case of dwarfism. In certain cases, hypothyroidism, hypopituitarism or other constitutional conditions will be found, developing as if by a trigger-like action following the onset of diabetes. One of the features of diabetes before the use of insulin was a great decrease in the B.M.R. under the influence of diets low in calories, possibly due to a depression of anterior pituitary function. Among our 29 cases are 2 in whom the possibility of disappearance or subsidence of diabetes exists. The first has continued to maintain his weight and strength and blood sugar has returned to normal, although the blood sugar had been abnormal at a glucose tolerance test 6 years ago. The second was the third case in Cushing's series, a man who had had a good deal of sugar in the urine but upon his return in his final illness, his urine was sugar free. However, at this time he had auricular fibrillation and cardiac failure with much edema. It is not rare to observe that in adult patients with mild diabetes, development of cardiac failure with much edema leads to disappearance of sugar from the urine and return of the blood sugar to normal, practically a remission, in diabetes. Possibly one may argue that when this occurs in an ordinary diabetes it is an evidence of the pituitary character of diabetes, for which assumption no proof yet exists. The fact should be emphasized that diabetes, when it exists with acromegaly or with pituitary disease, is still true diabetes and requires the same type of treatment as other cases of similar severity. Twenty-five per cent

of the cases referred to by Cushing died in diabetic coma. In our present series of 29 cases, gangrene and amputation of a leg has occurred in 1 case, coma in 3 cases, severe infection of a leg requiring surgical treatment in 1 case. Dietary treatment of the diabetes is difficult because often the patient has extreme hunger which will not permit restriction of diet. Sensitiveness to insulin varies in the acromegalic cases just as it does in other diabetic patients. It has often been stated that the implantation of an endocrine organ will not be successful unless there is a real deficiency of specific hormone in that patient. One might suppose that if a patient had a normal pancreas and then developed acromegaly with at first merely a functional strain on the pancreas that the addition of insulin would have little effect. Comparison might be made with illustrations of thyroid extracts. In a normal individual without any deficiency, a considerable dose of thyroid extract can be taken without making any appreciable change in pulse rate or the feelings of the patient whose thyroid has been removed, and yet in a patient of this type ½ to 1 grain a day is often sufficient to maintain metabolism at a level only slightly below normal.

Diabetogenic extracts of anterior pituitary. Evans (41) produced persistent glycosuria and hyperglycemia in dogs by means of injections of an anterior pituitary extract. He pointed out the necessity for some constitutional factor, since of 2 litter mates so treated, one developed and the other did not develop glycosuria. Evans (44) also succeeded with such an extract in producing not only glycosuria and hyperglycemia in dogs, but actual acidosis and coma, from which recovery was brought about by means of insulin. Young (1) improved the method of preparation of the anterior pituitary extract and produced a chronic diabetes in a dog by injections of relatively large amounts of an extract of anterior pituitary lobe of oxen. Best (2) showed that when such a dog's pancreas was removed, the diabetes was not made more severe as would have been expected had the effect of the injection of the anterior pituitary extract been exerted solely outside the pancreas. Furthermore, the pancreas of the dog contained almost no insulin. Upon pathological examination, the islands of Langerhans showed hydropic degeneration. Later, Young was able to produce all stages of change in the islands of Langerhans by varying the size and the duration of the injections with anterior pituitary extract. So far, no signs of acromegaly in the animals have appeared. Either the period of injection is too short or the growth hormone is absent or inactive. The activity of the extract varies in different species. Already Young has found evidence that the extract may contain two hormones affecting the islands of Langerhans. The one may be pancreatropic, as evidenced by the appearance of hypertrophied islands of Langerhans, at certain stages of the injections. The other may be diabetogenic, as it produces finally degeneration of the islands. The hope is afforded that eventually a pancreatropic hormone may be separated.

SUMMARY

Twenty-nine cases of combined acromegaly and diabetes are reviewed. Excessive height rarely occurred. The average interval between the onset of acromegaly and that of diabetes was 9.2 years. A comparison of the symptoms in diabetes and acromegaly shows certain similarities. A constitutional or hereditary predisposition to diabetes underlying the diabetes encountered in acromegaly is suggested by the existence of hereditary or familial diabetes in 6 of the 29 cases, or 21%; whereas, among relatives of 127 acromegalics without known diabetes, only 2% had diabetes. Obesity in 113 acromegalics resembled that noted in the histories of diabetics in that 73% of the acromegalics had been from 10 to 70% above standard weight.

Absence of the specific dynamic action of protein was observed in one case of

acute hyperpituitarism at the beginning of a period of diabetic treatment; a subsequent test after diabetic treatment showed a normal specific dynamic action. During this period the basal metabolism fell from +34% to +1%.

Comparison was made of the weights of organs at autopsy between groups of patients with acromegaly, diabetes, combined acromegaly and diabetes and Simmonds' disease. Splanchnomegaly occurred only in the presence of acromegaly. Its absence in cases of ordinary diabetes argues against the possibility that persistent hyperpituitarism caused the diabetes. The possibility that a brief period of acute hyperpituitarism could produce permanent damage to the islands of Langerhans without splanchnomegaly remains. However, 4 cases of fugitive acromegaly due to mixed tumors of the pituitary did not develop diabetes. In 50 diabetic autopsies variations in the size of the pancreas were not associated with variations in the weight of other internal organs, such as was shown by autopsies of cases of acromegaly and of Simmonds' disease.

The clinical character of the diabetes associated with acromegaly does not show any greater variation in severity, resistance to insulin, and duration of life than is seen in a large group of diabetics without acromegaly. The inconsistency between a blood sugar curve indicating insulin insensitivity and the clinical fact that the patient had repeated insulin reactions was observed. The usual complications of diabetes including pyogenic infections, arteriosclerosis with gangrene and coronary atherosclerosis occurred.

Acromegalic diabetics may develop coma and therefore require the same careful adjustment of diet and insulin as non-acromegalic diabetics of similar severity. In certain mild cases of acromegaly and diabetes excessive polyphagia without loss of weight or development of acidosis resembled the diabetes produced by Young by the injection of anterior pituitary extract. The demonstration by Young that degeneration of the islands of Langerhans and chronic diabetes can be produced in the dog by injection of large amounts of crude anterior pituitary extract of the cow provides a new method of studying the possible production of such changes in the human diabetic by such a diabetogenic hormone.

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A COMPARATIVE STUDY OF THE METABOLIC EFFECTS OF TESTOSTERONE PROPIONATE IN NORMAL MEN AND WOMEN AND IN EUNUCHOIDISM[1,2]

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LAST YEAR, in conjunction with A. H. Bryan, we described our experiences with the metabolic effects of daily injections of testosterone propionate in 4 eunuchoids, one of whom had pituitary damage from a suprasellar cyst (1, 2). There appeared a uniform decline in urinary nitrogen reflected in the urea fraction and unaccompanied by increase in the plasma protein, non-protein nitrogen, urea or hemoglobin concentrations in the blood; a decline in urinary sodium associated, except in one instance, with a somewhat smaller decline in urinary chloride; a slight decline in urinary potassium in 2 experiments, of which one was fragmentary; a reduction in urine volume and a gain in weight due chiefly to water held with the retained electrolytes and nitrogen. Urinary creatinine remained unaffected while creatine declined in the 2 experiments in which spontaneous creatinuria was sufficiently intense and sustained to permit satisfactory study. The 3 patients without known pituitary disease, together with a fourth examined previously (3), showed slight rises in the basal metabolic rates. In conjunction with W. H. Hoskins and J. R. Coffman we showed that urinary androgens rose from subnormal levels to values usually within the upper reaches of the normal, and urinary estrogens, likewise increasing, attained the normal average (4). Within a few days after discontinuing testosterone propionate all affected processes moved well in the direction of the pretreatment baselines. It was pointed out that the amounts of protein estimated as stored, from 109 to 395 gm. in 10 to 15 days, were far in excess of those conceivably going to enlarging genital accessories, thus indicating deposit of new protein elsewhere in the body. The retention of sodium, chloride and associated water was held to be analogous to that produced by the chemically related adrenal cortical substances in men and dogs with intact adrenals (5–9), an analogy well supported by the demonstration of Thorn and Harrop (10), of the 'sodium retaining' properties of estrone, estradiol, progesterone, pregnandiol and testosterone and its propionate, all of the steroid family, in the normal dog. This analogy with the adrenal cortical substance failed, however, with respect to urinary potassium excretion since this usually increases after these steroids are administered to the normal organism rather than diminishing as in our eunuchoids after testosterone propionate. The dosage used in our work, 25 mg. of testosterone propionate daily, was quite sufficient in protracted

[1] The substance of this paper was read before the Association for the Study of Internal Secretions, May 12, 1939.

[2] This study was supported in part by grants from the Rockefeller Foundation.

experiments to cause enlargement of the penis and prostate, deepen the voice and increase the body hair of underdeveloped men (3).

It is now important to consider the response of the normal organism, already under the influence of its own androgens, to administered testosterone propionate. Kochakian and Murlin (11, 12) and Kochakian (13), who first described the nitrogen retaining properties of urinary androgens, androstene-dione and testosterone and its esters in the castrate dog, emphasized that there was a maximal effect in the neighborhood of 0.05 gm. of nitrogen stored per kg. per day which could not be exceeded by increasing or protracting the dosage. In one normal dog (12), they were unable to influence urinary nitrogen by 140 mg. of androstene-dione given in 3 portions in 4 days, the animal being followed for 11 days after the last injection. They considered, therefore, that the normal dog is at his maximum capacity for response by virtue of the influence of the hormones from his own testes. Thorn and Engel (14), however, have noted a decline in urinary nitrogen of as much as 4 gm. per day (0.4 gm. per kg. per day) after 25 mg. of testosterone propionate daily for 7 days in a normal dog. The discrepancies between these 2 experiments may lie in the androgen used, in dosage or in the state of gonadal function of the animals involved.

It now appears from the studies of Thorn and Engel (14) that the normal dog is quite insensitive to the 'sodium and chloride retaining' influence of testosterone propionate, daily injections of 25 mg. for 7 days producing no change whatever in the urinary excretion of these electrolytes or of water, although, as described above, substantial nitrogen retention did occur, accompanied by reduction in the urinary excretion of inorganic phosphorus and potassium. In an impotent man, otherwise normal (15), however, these workers (14) obtained a maximum reduction in urinary sodium excretion of about 30 m.eq. per day (ave. decline 19), a decline in urine volume and a gain in weight of 0.6 kg., together with a maximum decline in urinary potassium of about 7 m.eq. per day (ave. decline 5), a maximum decline in urinary inorganic phosphate of about 0.22 gm. per day (ave. decline 0.18) and a maximum decline in total urinary nitrogen of about 1.5 gm. per day (ave. decline 1.2), after giving 25 mg. of testosterone propionate intramuscularly for 6 days. The direction of these changes is in agreement with our experience in the eunuchoids, although the degree of change is in certain instances not as great, a point of some interest to be discussed at length later.

The present communication deals with an extension of our studies on the metabolic effects of testosterone propionate[3] in eunuchoidism and a comparison of these effects with those obtained in 2 normal men and 2 normal women. One experiment on the effect of testosterone,[4] by inunction, on a eunuchoid is included.

METHODS

The subjects were placed upon weighed diets containing the same food each day. The food was not analyzed, but reasonable constancy of intake was secured by (*a*) serving each individual slices from a single 20 lb. sirloin butt, which lasted about 25 days, supplemented by one or more such butts as necessary for the longer experiments; (*b*) serving the cooked foods and butter salt-free and adding measured amounts of salt at the table; (*c*) using canned fruits and vegetables largely, and these from the same case for each experiment, as far as possible; (*d*) by each patient eating all of his food each day. One to two weeks of the constant diet always preceded the beginning of collections. Caloric content was estimated to provide maintenance of weight with sufficient energy for a busy but regular day's work, as all of our normals continued their usual activities as students or as laboratory technicians, and our

[3,4] The testosterone propionate and testosterone ointment were provided through the kindness of Drs. Gregory Stragnell, Erwin Schwenk and Max Gilbert of the Schering Corp., Bloomfield, N. J.

patient, *J.X.*, occupied himself about the hospital and its environs. A. H. Bryan, in conjunction with ourselves, showed previously (2) that the characteristic effects of testosterone propionate are observed while the total caloric expenditure, as measured by the insensible loss of weight, remains essentially unchanged. The fluid intake was constant and sweating did not occur. In the second experiment on *J.X.* (fig. 1), 1.32 gm. of creatine hydrate (1 gm. of creatine) was given daily by mouth to insure sufficient creatinuria for study.

These conditions permit maintenance of daily urinary excretion of nitrogen with maximum deviations of 0.3 to 0.7 gm. from the mean, inorganic phosphorus with maximum deviations of 0.02 to 0.1 gm., and potassium usually with maximum deviations well under 10 m. eq.; but sodium and chloride may on occasional days deviate as much as 25 m. eq. from the average control value, without known errors in technic and without obvious explanation. Thorn and his coworkers (16) noted the same tendency for spontaneous and unexplained variations in sodium and chloride excretion in a normal untreated man.

For the most part, all of the urine passed was collected in containers to which just enough 5 N sulfuric acid had been added to insure acidity. In order to permit determinations of pH, etc., in the third experiment on the eunuchoid *J.X.*, all urine reaching the bladder between 10:00 P.M. and 7:00 A.M. was voided at one time through a funnel, the stem of which dipped beneath oil in a clean cylinder. The remainder of the urine for these days was handled in the usual manner. During this experiment drinking water was given in specified amounts at specified times so as to minimize variations in urine volume during each period.

Analyses were made on the urine from each portion of the divided days of the third experiment on *J.X.*, otherwise on 24-hour specimens, or as in the normal men, *R.W.* and *J.L.*, on urine combined into 2-day lots. Chemical determinations were made in the clinical chemistry laboratory of the Department of Medicine by procedures given in Peters and Van Slyke (17), except where otherwise noted: sodium in urine and serum, Butler and Tuthill as modified by Eichelberger (18); chloride in urine and serum, Wilson and Ball; potassium in urine and serum, Shohl and Bennett as modified by Eichelberger (18);[5] inorganic phosphorus in urine and serum, Fiske and Subbarow (19); urine pH by the glass electrode; total CO_2 in urine, Van Slyke manometric; titratable acidity of urine, Folin, to the endpoint of phenolphthalein; ammonia N in urine, Folin permutit with nesslerization; urea N in whole blood, gasometric urease method of Van Slyke; N.P.N. in whole blood and plasma, Koch and McMeekin as given by Koch (20); total N in urine and plasma, macrokjeldahl, distilling the ammonia formed into boric acid solution, according to the procedure described by Meeker and Wagner (21) and titrating to the brom cresol green endpoint; plasma proteins from (total N-N.P.N.) x 6.25; creatine and creatinine in urine, Folin (creatine by autoclave); cholesterol in whole blood, Lieberman-Burchard colorimetric determination in Bloor's alcohol-ether extract. Hemoglobin was determined by Newcomer and cell volume by hematocrit.

Basal metabolic rate determinations were made by the Benedict-Roth method on *J.X.* and *E.S.* and by the gasometer method (22) on *P.J.*, *R.W.* and *J.L.* The Mayo Foundation standards (23) were used for comparison.

The androgen assays were performed in the Department of Biochemistry by W. H. Hoskins, J. R. Coffman and G. W. Beach and will be published in detail subsequently. Through the courtesy of these collaborators we are able to refer in our discussion to their results on the pooled urine during the control periods. The urine was kept in the refrigerator and extracted within the week following the termination of collections. After hydrolysis by boiling 15 minutes with hydrochloric acid, as described by Peterson, Gallagher and Koch (24), it was extracted with benzene in a continuous extractor, separated into androgenic and estrogenic fractions by partition between ether and 10% aqueous sodium hydroxide and the androgenic, ether-soluble fraction assayed on 7 capons by the method of Gallagher and Koch (25). The entire procedure is given in detail by Gallagher, et al. (26).

PROTOCOLS

J.X. (referred by Dr. Carl R. Moore), was a 27-year-old eunuchoid with bilaterally

[5] This method was also used in our previous work instead of that of Clausen, Kramer and Tisdall as was erroneously stated in the paper (2).

TABLE 1. THE EFFECT OF TESTOSTERONE BY INUNCTION ON THE EXCRETION OF URINARY NITROGEN AND ON THE BODY WEIGHT OF THE EUNUCHOID, J.X. (FIRST EXPERIMENT)

Date	Procedure	Urinary N per day		Estimated N retention[1]		Change in body weight
		Average	Range	Per day	Total	
		gm.	gm.	gm.	gm.	kg.
8/13 to 18	Control	7.63	7.04–7.94			−0.3
8/19 to 27	Inunction (25 mg. testosterone daily)	7.04	6.60–7.38	0.59	5.31	−0.4
8/28 to 9/9	Inunction (50 mg. testosterone daily through 9/5)	6.33	5.36–6.94	1.30	15.60	+0.1
9/10 to 20	Recovery	6.75	5.61–7.26	0.88	9.68	−0.5
9/21 to 29	Control	7.42	6.79–7.84			−0.2

[1] The estimated retentions are based upon subtracting the average urinary nitrogen excretion for a given period from the average urinary excretion during the initial control period.

undescended testes who entered the Albert Merritt Billings Memorial Hospital August 2, 1938. Surgical treatment at the age of 9, and subsequent treatment with pregnancy urine extract had failed to effect descent of the testes. At the time of his admission he was 180 cm. tall and weighed 63 kg. His maximum weight had been 77 kg. 2 years ago. There was a suprapubic fat pad and considerable deposit of fat about the hips giving them a feminine contour, but the breast areas had no unusual fat deposits. His face was smooth, boyish and devoid of terminal hair. Axillary hair was present but not abundant and pubic hair was scant. Limb and body hair was scant and fine. His voice was boyish and the laryngeal cartilage did not possess the characteristic masculine prominence. His testes could not be felt and the empty scrotum was small but well differentiated. The penis was 4.2 cm. long and 1.9 cm. wide at the corona. A very small amount of Y-shaped prostatic tissue was felt about the urethra. Erections occurred infrequently and sexual inclinations toward women were not recognized by the patient.

His initial B.M.R. was −28. X-rays showed a normal sella turcica. The epiphyses about the shoulder, elbow and hand were closed, those of the knee and distal ends of the radius and ulna were fusing. Visual fields were normal. The result of a glucose tolerance test was normal. Wassermann and Kahn test results were negative; Hb. was 13.2 gm. % (Newcomer);

TABLE 2. THE EFFECT OF TESTOSTERONE PROPIONATE, 25 MG. DAILY ON CERTAIN BLOOD CONSTITUENTS OF THE EUNUCHOID, J.X. (SECOND EXPERIMENT)

Date	Plasma proteins	N.P.N. of plasma	Urea N of whole blood	Hb.	R.B.C.	R.B.C.	Chol. of whole blood
	gm./100 cc.	mg./100 cc.	mg./100 cc.	gm./100 cc.	m./cu. m.m.	vol. %	mg./100 cc.
9/20/38	7.03	17	7.0	13.7	3.95	37	166
9/22	7.26	16	7.6	13.6	3.98	37	180
9/27	6.99	20	9.6	13.9	4.04	36	167
9/29	6.72	17	9.3	13.6	3.87	37	163
10/4	6.96	14	6.2	14.1	3.96	38	161
10/6	7.19	15	6.9	14.1	4.01	38	
10/11	6.74	15	6.2	14.4	4.10	38	155
10/13	6.16	11	4.2	14.1	4.01	38	
10/18	7.07	20	8.3	13.9	4.03	38	152
10/20	7.38	17	7.2	14.1	4.15	39	172
10/26	7.73	16	8.7	14.2	4.16	37	173
11/1	8.18	18	9.7				
11/3	7.26	18	8.8				178
11/8	6.72	19	9.8				161
11/11	6.94	20	11.0				157

Testosterone propionate was given intramuscularly from September 30 through October 13.

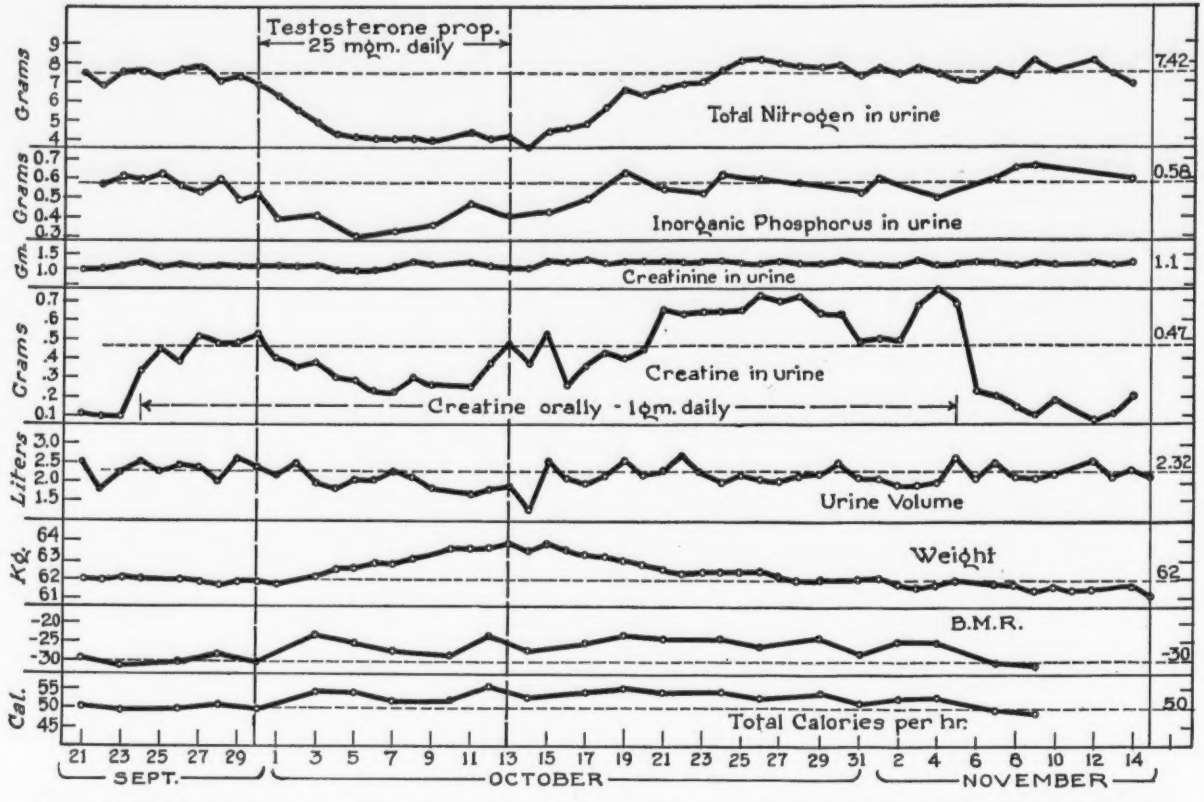


Fig. 1. The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents, body weight and BM.R. of the eunuchoid J.X. (second experiment). Treatment was given from September 30 through October 13.

TABLE 3. THE EFFECTS OF TESTOSTERONE PROPIONATE (50 MG. DAILY), ON THE COMPOSITION OF THE NIGHT URINE OF THE EUNUCHOID, J.X. (3RD EXPERIMENT)

Date	Vol. cc.	pH	$BHCO_3$ mM.	$BHCO_3$ mM.	Titratable Acid m.eq.	Titratable Acid m.eq.	Inorganic Phosphorus mM.	Inorganic Phosphorus mM.	Ammonia N mM.	Sodium m.eq.	Chloride m.eq.	Total N gm.
			period	liter	period	liter	period	liter	period	period	period	period
11/16	520	5.48	0.24	0.46	10.5	20.2	7.1	13.7	—	9.1	5.3	2.95
11/17	270	5.25	0.10	0.38	9.1	33.6	5.2	19.3	9	4.8	3.4	2.24
11/20	440	5.74	0.51	1.17	9.3	21.2	6.5	14.8	—	13.1	7.9	2.52
11/21	330	5.07	0.04	0.11	10.2	31.0	6.1	18.5	11	3.9	3.4	2.36
11/22	410	5.31	0.09	0.22	9.6	23.3	6.1	14.9	8	7.0	4.5	2.50
11/24	350	5.35	0.10	0.30	9.1	25.9	5.8	16.6	—	4.8	4.5	2.07
11/25	370	5.39	0.10	0.27	9.1	24.6	6.1	16.5	—	4.8	4.2	1.87
11/27	240	5.57	0.10	0.43	6.6	27.4	3.9	16.3	8	1.7	2.8	1.34
11/28	240	5.65	0.14	0.57	5.7	23.9	3.2	13.3	8	0.9	2.8	1.33
11/29	260	5.56	—	—	6.0	23.0	3.2	12.3	6	1.3	2.3	1.26
12/ 2	450	6.23	1.30	2.89	7.1	15.7	6.1	13.6	7	16.1	8.7	1.66
12/ 4	450	5.94	0.74	1.65	8.8	19.6	6.5	14.4	6	16.1	9.0	1.86
12/ 6	440	5.05	0.06	0.13	11.1	25.2	7.7	17.5	7	10.4	6.5	2.24

Testosterone propionate was given daily in 2 equal intramuscular injections from 11/23 through 11/27. The effects lasted some 3 days after the last injection. Each night period given represents urine for 9 hours, voided at one time in the morning. The creatinine excretion for this period was constant throughout the experiment. Titratable acidity is given to the endpoint of phenolphthalein.

r.b.c. 3,930,000 per cu. mm.; cell volume 38%; w.b.c. 4,700 per cu. mm., with a normal differential count.

Mr. John Evans of the Department of Biochemistry found about 8 mouse units of gonadotropic material in the benzoic acid precipitate of a 24-hour urine specimen tested upon the immature mouse.

TABLE 4. THE EFFECT OF TESTOSTERONE PROPIONATE (25 MG. DAILY) ON CERTAIN BLOOD CONSTITUENTS OF THE NORMAL MEN, R.W. AND J.L.

Date	N.P.N. in whole blood	Urea N in whole blood	P (inorg.) in serum	Na in serum	Cl in serum	K in serum
	mg./100 cc.	*mg./100 cc.*	*mg./100 cc.*	*m.eq./liter*	*m.eq./liter*	*m.eq./liter*
			R.W.			
2/28/39	26		4.1	142.5	102.6	4.21
3/ 2	26		4.4	142.1	97.2	4.65
3/ 7	24		4.2	140.6	100.2	4.55
3/ 9	23		4.0	140.1	102.8	4.16
3/14	28		3.9	139.1	104.2	4.55
3/16	21		3.5	140.6	100.8	4.16
3/21	30		5.0	138.0	101.2	5.00
3/23	30		4.2		99.4	4.50
			J.L.			
3/23/39		11.7	4.2			
3/25		13.5	3.8			
3/30		11.5	4.2			
4/ 3		11.1	4.0			
4/ 6		11.8	3.7			
4/10		8.3	3.5			
4/14		11.8	4.5			
4/17		10.0	4.0			

Testosterone propionate was given intramuscularly from March 6 through March 15 in *R. W.*, and from April 2 through April 9 in *J. L.* The effects were manifest for some two days after the last injection in each subject.

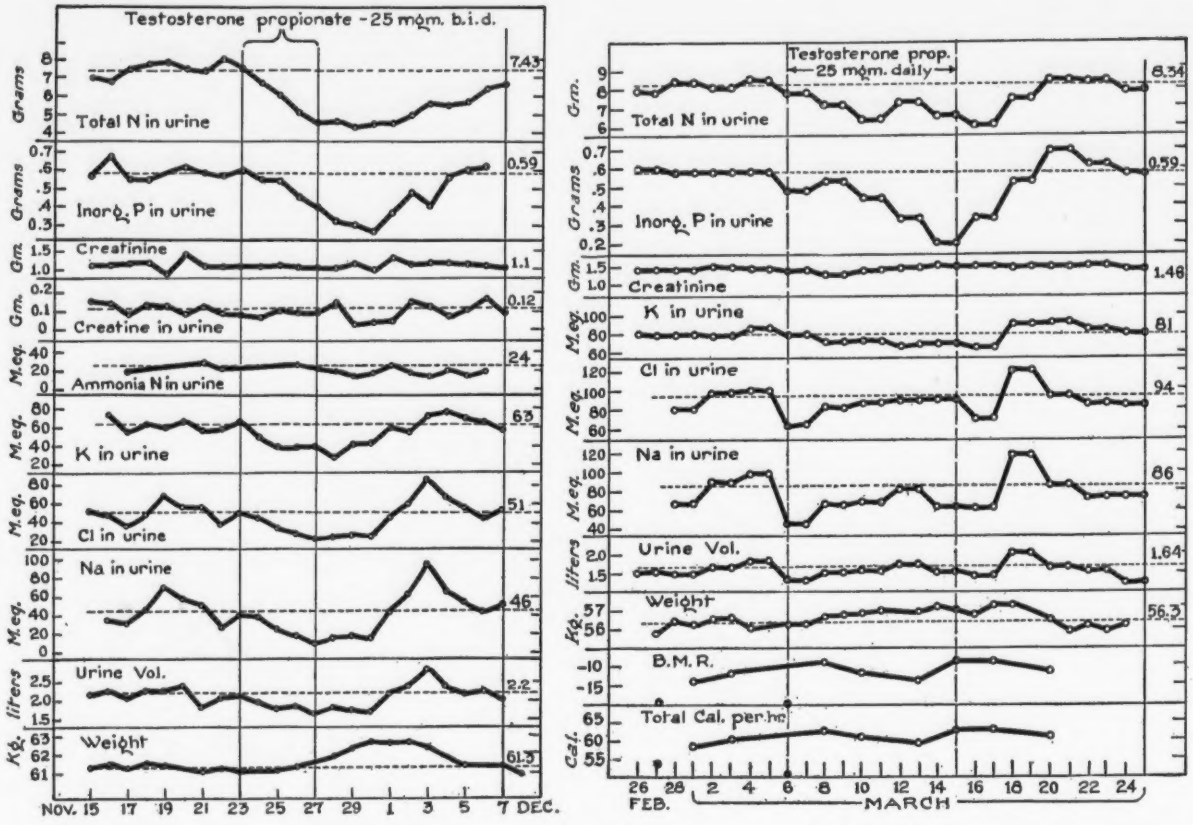


Fig. 2. (left). The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents and body weight of the eunuchoid J.X. (third experiment). Treatment was given from November 23 through November 27. Fig. 3 (right) The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents, body weight and B.M.R. of the normal man R.W. Treatment was given from March 6 through March 15. The subject fell asleep during the determination of the B.M.R. on Feb. 27 and March 6, and the somewhat lower values for these days are hence recorded as isolated points.

On August 8 the patient was placed upon a constant diet of carbohydrate 267, protein 48, fat 100, Cal. 2,160, food water 1,580, drinking water 1,000, added salt 2 gm., giving an estimated content of N 7.7 grams, P 1.12 grams, Na 62 m. eq., K 83 m. eq., Cl 63 m. eq. as judged from standard food tables. He endured this diet with inexhaustible patience throughout the entire extent of his hospital stay. He was allowed to be up and about the hospital and campus each day.

Experimental observations were divided into 3 periods. From August 19 to and including September 5, testosterone in an ointment was rubbed into the skin of the trunk, chest and thighs, providing 25 mg. daily of testosterone from August 19 to 27, and 50 mg. daily from August 28 to September 5. In the 18 days of this experiment there was slight physical change.

TABLE 5. COMPARISON OF THE ESTIMATED REDUCTION IN EXCRETION OF SEVERAL URINARY CONSTITUENTS PRODUCED BY TESTOSTERONE PROPIONATE, GIVEN INTRAMUSCULARLY TO HYPOGONAD AND NORMAL MEN AND TO NORMAL WOMEN

Subject	Age	Condition	Urinary androgens before treatment	Amount of test. prop. given	Average reduction in excretion of urinary constituents						
					Total Nitrogen			Inorganic phosphorus	Sodium	Chloride	Potassium
					I	Maximal* II	Maximal* III				
			I.U. per day	mg. per day	gm./day	gm./day	gm./kg. per day	gm. per day	m.eq. per day	m.eq. per day	m.eq. per day
F.R.	27	Eunuchoid	13	25	4.51	5.14	.069		24	19	
M.D.	51	Eunuchoid	10	25	2.12	3.19	.064		17	7	
N.D.	31	Eunuchoid	3	25	2.39	3.03	.057		15	(−11)	9(4 dys only)
H.S.	24	Eunuchoid Hypopituitary	2	25	1.16	1.59	.025		14	6	7
J.X. 2	27	Eunuchoid	29, 37	25	2.80	3.27	.053	.170			
3		Eunuchoid		50	2.14	2.93	.048	.150	23	18	20
R.W.	21	Normal Man	83	25	1.37	1.66	.029	.190	21	15	9
J.L.	19	Normal Man	78	25	1.89	2.55	.030	.050	28	33	12
P.J.	24	Normal Woman	100	25	2.10	2.61	.043	.150	9	5	9
E.S.	31	Normal Woman	65	25	.56	.73	.016	.990	3	5	4

The values given for reduction in urinary excretion of various constituents are obtained by subtracting the average daily excretion during the 'experimental' period from the average pre-treatment level. The 'experimental' period thus used (excepting columns II and III under nitrogen) extends from the beginning of treatment to a point just before the onset of recovery, a point which occurs, as a rule, 2 or 3 days after the last injection of testosterone propionate.

* The 'experimental' periods covered in columns II and III under nitrogen extend from the time of approximately maximal effect of the androgen to just before the beginning of recovery. The data given for *F. R.*, *M. D.*, *N. D.*, and *H. S.* are from previous publications (2, 4).

Toward the end he had several erections and the penis, then 5.0 cm. long, and the scrotum were slightly hyperemic. The prostate was unchanged. Data on nitrogen excretion and body weight are given in table 1.

One and thirty-two hundredths grams of creatine hydrate (1.00 gm. of creatine) were given orally in one dose daily from September 24 to and including November 5 to insure creatinuria. From September 30 to and including October 13 (14 days) he received 25 mg. daily of testosterone propionate intramuscularly in sesame oil. During the course of this treatment erections increased somewhat in frequency but were at no time as strong, frequent and troublesome as they may be, on occasion, with such medication. The hyperemia of the penis and scrotum returned and the penis increased in length from 4.2 cm. to 5.8 cm., and the corona in width from 2.1 to 2.5 cm.; the prostate seemed slightly larger to several observers. The metabolic data, accumulated during this study are given in figure 1 and table 6, and that on certain blood constituents in table 2.

K.W.: Treatment was given from March 6 through March 15. The subject fell asleep during the determination of the B.M.R. on Feb. 27 and March 6, and the somewhat lower values for these days are hence recorded as isolated points.

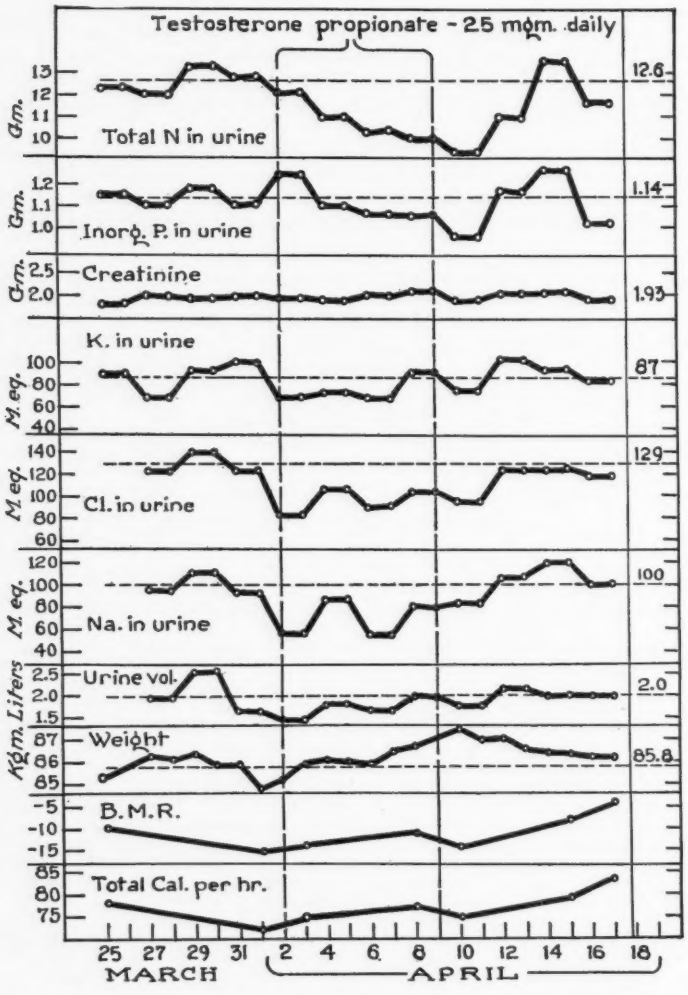


Fig. 4. The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents, body weight and B.M.R. of the normal man *J. L.* Treatment was given from April 2 through April 9.

From November 23 to and including November 27 (5 days) he received 50 mg. of testosterone propionate, divided into 2 equal intramuscular injections in sesame oil. For several of the days of this experiment, each 24-hour period was divided into a night and day portion as described under methods. The data on the chemical studies of this urine are given in figure 2 and table 3.

R.W. and *J.L.* were normal young unmarried men, university students, age 20 and 19 respectively. They contrasted strongly in physical type. *R.W.* was of linear build and *J.L.* was heavy and broad. *R.W.* was blond and lightly bearded, requiring shaving only every other day, while *J.L.* was heavily bearded and had a deeper voice. The composition of the constant diets was for *R.W.*: carbohydrate 379, protein 60, fat 105, Cal. 2,705, food water

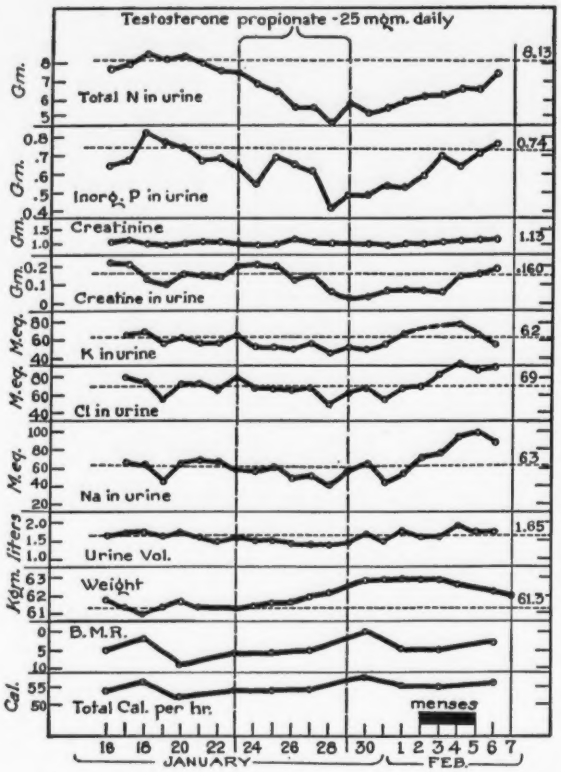
1,490, drinking water 1,000, added salt 4 gm.; with an estimated composition of N 9.6 gm., P 1.27 gm., Na 100 m. eq., Cl 100 m. eq., K 118 m. eq. For *J.L.* the diet was carbohydrate 443, protein 89, fat 143, Cal. 3,415, food water 2,070, drinking water 1,300, added salt 4 gm.; with an estimated composition of N 14.2 gm., P 1.76 gm., Na 112 m. eq., Cl 114 m. eq., K 121 m. eq. Both pursued their usual activities as students during the course of the experi-

TABLE 6. THE EFFECTS OF TESTOSTERONE PROPIONATE, GIVEN INTRAMUSCULARLY, ON THE B.M.R., FASTING RESPIRATORY QUOTIENT, PULSE RATE AND BLOOD PRESSURE OF NORMAL MEN AND WOMEN AND OF THE EUNUCHOID *J.X.*

Date	Weight kg.	B.P. syst./diast.	Pulse Rate	R.Q.	B.M.R.	Total Cal. per hr.
R.W. Normal man, age 20, ht. 174 cm.						
2–20	55.0	104	68	66	0.75	− 9	61.5
21	56.3	100	62	66	0.78	− 8	62.8
24	56.3	100	62	59	0.83	−16	57.3
27	55.8	104	68	59	0.79	−20[1]	54.4[1]
3–1	56.3.	104	68	65	0.83	−14	59.2
3	56.6	106	58	68	0.86	−12	60.3
6	56.2	100	66	59	0.83	−22[1]	54.0[1]
8	56.6	102	60	70	0.81	− 9	62.6
10	56.8	102	66	67	0.82	−12	60.6
13	56.9	92	50	65	0.82	−14	59.2
15	57.0	102	66	71	0.86	− 9	62.5
17	57.2	104	64	69	0.83	− 9	62.5
20	56.4	102	60	66	0.84	−12	60.4
25 mg. testost. prop. daily March 6–15 inclusive. [1] Asleep?						
J.L. Normal man, age 19, ht. 181 cm.						
3–14	86.8	114	78	70	0.83	− 3	83.8
21	87.0	102	60	61	0.83	−12	76.3
23	86.3	102	62	60	0.86	−16	72.7
25	85.4	98	62	63	0.82	− 9	78.5
4–1	84.8	108	72	62	0.79	−15	72.6
3	85.9	102	68	60	0.83	−14	74.7
8	86.7	102	66	67	0.85	−11	77.0
10	87.4	108	70	66	0.84	−14	75.2
15	86.3	106	66	76	0.86	− 8	79.5
17	86.2	102	66	65	0.87	− 4	83.3
25 mg. testost. prop. daily March 2–April 9, inclusive.						
J.X. Eunuchoid, age 27, ht. 180 cm.						
9–21	62.1	102	62	44		−29	50.6
23	62.1	102	62	45		−31	48.9
26	62.0	102	72	43		−30	50.0
28	61.8	102	72	45		−28	51.2
30	61.9	102	62	45		−30	49.7
10–3	62.2	104	74	46		−23	54.6
5	62.7	104	64	52		−25	53.9
7	62.9	100	70	51		−27	52.3
10	63.7	102	72	50		−28	51.9
12	63.7	92	72	51		−23	55.6
14	63.6	92	62	51		−27	52.5
17	63.4	102	62	47		−25	53.9
19	63.1	98	64	42		−23	54.8
21	62.6	116	56	46		−24	54.0
24	62.5	94	54	46		−24	54.0
26	62.5	98	68	44		−26	52.7
29	62.1	102	72	45		−24	53.9
31	62.0	94	64	45		−28	51.6
11–2	61.9	92	62	44		−25	51.6
4	61.8	98	68	43		−25	53.3
7	61.9	102	72	44		−30	50.0
9	61.5	98	68	44		−31	48.8
25 mg. testost. prop. daily Sept. 30–Oct. 13 inclusive.						

Date	Weight	B.P. syst./diast.	Pulse Rate	R.Q.	B.M.R.	Total Cal. per hr.
P.J. Normal woman, age 24, ht. 155 cm.						
1–11	61.6	118	72	88	0.81	− 1	56.5
13	61.6	114	72	86	0.74	− 1	57.0
14	62.0	114	68	82	0.78	− 2	56.1
16	61.7	118	70	81	0.78	− 5	54.2
18	61.0	110	70	84	0.77	− 2	56.0
20	61.3	102	62	76	0.77	− 9	52.0
23	61.3	106	66	75	0.79	− 6	53.7
25	61.5	94	62	82	0.79	− 6	53.6
27	61.9	102	68	81	0.78	− 5	54.2
30	62.8	106	64	92	0.80	0	57.4
2–1	62.9	102	60	84	0.83	− 5	54.9
3	62.8	108	66	87	0.80	− 5	54.8
6	62.2	102	62	76	0.81	− 3	55.7
25 mg. testost. prop. daily Jan. 23–Jan. 29 inclusive. Menses 1–11 through 1–15, 2–2 through 2–5.						
E.S. Normal woman, age 31, ht. 165 cm.						
1–13	45.9	94	60	58		−16	44.1
14	45.4	92	54	55		−12	45.9
16	45.3	88	50	54		−18	42.6
17	45.3	94	58	55		−15	44.3
18	45.1	90	52	55		−16	43.7
20	45.4	84	48	57		−19	42.1
23	45.5	94	52	55		−17	43.4
25	45.8	90	52	54		−18	42.8
27	45.5	86	48	55		−14	45.2
30	45.8	94	60	54		−20	41.7
2–1	46.2	96	56	58		−13	45.8
3	46.8	90	56	59		−17	43.8
6	46.3	90	48	63		−15	44.8
8	46.3	88	48	59		−14	45.2
10	46.5	90	54	60		−21	41.7
13	46.2	88	52	56		−22	40.6
15	46.4	90	48	53		−21	42.0
25 mg. testost. prop. daily Jan. 29–Feb. 4 inclusive. Menses 1–16 through 1–19 and 2–13 through 2–16.						

ments. *R.W.* received 25 mg. of testosterone propionate daily in sesame oil intramuscularly, from March 6 to and including March 15, a total of 10 days. *J.L.* received 25 mg. of testosterone propionate similarly from April 2 to and including April 9, a total of 8 days. Neither was aware of any very distinct subjective response, although *R.W.* thought that erections might have occurred slightly more frequently and that a slight increased frequency of urination and tendency to contraction of the scrotal musculature were present during treatment.



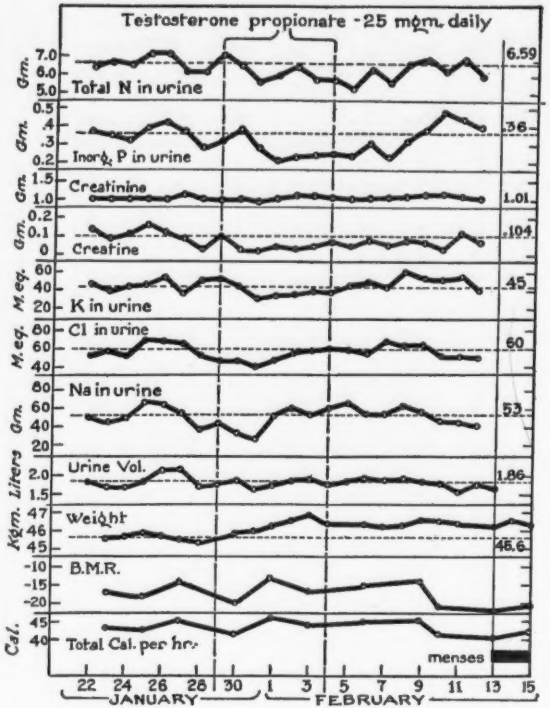


Fig. 5 (left). The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents, body weight and B.M.R. of the normal woman, *P.J.* Treatment was given from January 23 through January 29. The first day of the menstrual cycle studied was January 11. Fig. 6 (right). The effect of intramuscular injections of testosterone propionate on the excretion of several urinary constituents, body weight and B.M.R. of the normal woman, *E.S.* Treatment was given from January 29 through February 4. The first day of the menstrual cycle studied was January 16.

Both men noted a slump in sense of well being about two days after stopping the injections, this being more conspicuous in *R.W.*, and more striking than any stimulation observed during treatment. The data on these subjects is presented in figures 3 and 4, and in tables 4 and 6.

P.J. and *E.S.* were normal young unmarried women, laboratory technicians, age 24 and 30 respectively. They contrasted physically, *P.J.* being short and plump while *E.S.* was taller and slender. The menstrual histories were as follows: *P.J.* began at 12, menses occurred every 28 to 30 days and lasted 4 to 5 days. *E.S.* began at 14, menses occurred every 32 to 35 days and lasted 3 to 5 days. Neither gave a history of a gain in weight before the onset of the menstrual flow and daily weighings before, during, and after the menses preceding collections showed no such gain in weight. The composition of the constant diets was for *P.J.*: carbohydrate 357, protein 54, fat 96, Cal. 2,508, food water 1,370, drinking water 1,000 cc., added salt 3 gm.; giving an estimated content of N 8.64 gm., P 1.11 gm., Na 79 m. eq., K 102 m. eq., Cl 78 m. eq. For *E.S.* the diet was carbohydrate 330, protein 44, fat 68, Cal. 2,108, food water 960, drinking water 1,000 cc., added salt 3 gm.; giving an estimated content of N 7.04 gm., P .62 gm., Na 65 m. eq., K 74 m. eq., Cl 60 m. eq. Both pursued their usual work in the hospital laboratories throughout the experiments.

The menstrual period of *P.J.* preceding the experiment began on January 11, 1939, and lasted through January 15. She received 25 mg. of testosterone propionate in 1 cc. of sesame oil intramuscularly on January 23, and thereafter daily through January 29, in all for 7 days. On the fourth day after the last injection a fully normal menstrual flow started and lasted for 4 days. This bleeding appeared, therefore, 22 days after the onset of the last preceding period or 6 to 8 days earlier than usual. The subject believed, however, that she probably had had such intermenstrual intervals on rare occasions in the past, and one such occurred in May, 1939.

Determination of the protein content of the urine collected during menstruation showed not more than one-tenth gram of protein nitrogen, so that the data on all constituents for these days is given without correction for contamination with menstrual blood. The subject had no subjective response of any kind to the treatment. The metabolic data is shown in figure 5 and table 6.

The menstrual period of *E.S.* preceding the experiment began on January 16, 1939 and lasted through January 19. She received 25 mg. of testosterone propionate in 1 cc. of sesame oil, intramuscularly, on January 29 and thereafter daily through February 4, for 7 days in all. The next menstruation started on February 13, or 28 days after the onset of the last preceding flow, and was entirely normal. This intermenstrual interval was only some 4 days shorter than usual for this subject. As in *P.J.* there were no subjective effects of the treatment. The metabolic data is shown in figure 6 and table 6.

DISCUSSION

The response of the eunuchoid *J.X.* to the injection of testosterone propionate is in agreement with our previous experience, summarized in table 5. The characteristic precipitate decline in urinary nitrogen excretion is well seen, proceeding at the same rate with the 25 mg. daily dose (fig. 1) as with the 50 mg. (fig. 2), and reaching a fairly stable minimum in about 5 days. Such cumulation of active androgen as may be provided by continuing the 25 mg. dosage for as long as 14 days is without effect in further depressing nitrogen excretion. Experiment has not as yet defined limits to the duration of this influence of testosterone propionate. The data on *J.X.*, however show that very considerable previous accumulations of nitrogen in no way interfere with a perfectly satisfactory demonstration of the nitrogen retaining effect. Thus, under the influence of testosterone inunctions (table 1), this patient retained an estimated 30 gm. in 33 days, added 55.4 gm. in 25 days as a result of the first course of testosterone propionate injections, lost only 3.5 gm. of this, and then gained 30.7 gm. further in 16 days during the second course with the larger dose of testosterone propionate by injection, making a net gain of 112.6 gm. of nitrogen, 703.8 gm. calcu-

lated as protein, in 4 months of discontinuous treatment and continuous observation. The inconspicuous losses of retained nitrogen during recovery are well illustrated by the data given for the after-period in figure 1 and the fore-period in figure 2, comprising in all 38 days following the last injection of 25 mg. of testosterone propionate. This, together with a similar 21-day period in the eunuchoid *M.D.* (2), attests to the tenacity with which the nitrogen compounds formed under the influence of the androgen may be held in the body. It should be noted that the amounts of nitrogen retained in *J.X.* during the periods of maximum effects of testosterone propionate are of approximately the same order as in the other eunuchoids, although the androgen excretion in the urine before treatment was substantially higher (table 5).

As in *N.D.* (2, table 1), the concentrations of plasma protein and non-protein nitrogen and blood urea nitrogen and hemoglobin are not increased during treatment. Indeed toward the later phase of the testosterone propionate effect reductions of 44 to 50% in the concentrations of the non-protein and urea nitrogen are observed on one isolated day. Similar reductions of 41 to 43% occurred in *N.D.* but were not stressed because they were unconfirmed at that time. Both constituents return towards normal during recovery in both experiments.

Moore, Lamar and Beck (27) observed that testosterone in ointment produced a powerful androgenic effect on the genital accessories of the rat when applied to the skin. In accordance with this, is the definite if slight, decline in urinary nitrogen produced in *J.X.* (table 1) with 25 mg. of testosterone applied to the skin daily and a more considerable decline with 50 mg. daily. There was no clear cut effect on body weight or on the prostate gland. Slight hyperemia and enlargement of the penis appeared toward the end of the experiment. In general the effects were much less than when the propionate was given intramuscularly, but as we have not tested the propionate by inunction direct comparisons are difficult.

The urinary excretion of inorganic phosphorus declines sharply after testosterone propionate (fig. 1, 2). The reduction of urine volume and of urinary sodium and chloride excretion and the gain in body weight in *J.X.* after 50 mg. of testosterone propionate (fig. 2), are of about the same magnitude as in previous experiments (2), (table 5) after 25 mg. daily, and the same sharp diuresis with loss of weight appears during recovery. Fortunately the reduction in urinary potassium, which was slight in previous experiments, is convincingly shown in figure 2, the estimated 'retention' reaching the considerable value of an average of 20 m. eq. per day. Although all of these various affected urinary constituents proceed more or less together during the development of the testosterone propionate effect, their behavior differs in an interesting manner during recovery. Thus sodium and chloride, deposited largely in extracellular spaces, are quickly lost shortly after treatment is stopped, while nitrogen, presumably stored as protein within cells, is still being retained. The excretion of inorganic phosphorus and potassium rises more rapidly to the baseline than does that of nitrogen, but, as would be expected from their intracellular deposition in the organism, these substances are not as rapidly or as completely discharged as are sodium and chloride.

The data on *R.W.* (table 4), a normal man, in the course of whose treatment much the same sequence of events took place as in the eunuchoids, show that the concentrations of sodium, chloride and potassium in the serum are unaffected during these times of changing electrolyte excretion. This is true, likewise, when adrenal cortical extracts are given to normal man (5). The concentration of inorganic phosphorus in the serum, however, in both *R.W.* and *J.L.* seems to decline somewhat towards the end of the testosterone propionate effect, to rise thereafter.

The fluid retained under the influence of testosterone propionate causes no blood

dilution, as evidenced by the constancy of plasma protein concentration, red blood cell count, and cell volume in *J.X.* (table 2), and in *N.D.* (2, table 1). Barring an as yet undemonstrated increase in circulating blood volume, this fluid, which makes up the greater part of the weight gain, is distributed between intracellular and extracellular extravascular compartments. More complete balance studies than we have at hand are required to assign the proper proportion to each repository.

In the normal dog Hartman, Lewis and Toby (8) have shown that ammonia excretion rises as sodium excretion declines after the administration of adrenal cortical extract, the pH of the urine remaining unchanged, and Stahl, Atchley and Loeb (28) reported falling ammonia excretion during sodium diuresis at the onset of suprarenal insufficiency in the dog. Such observations constitute the basis for the often quoted, but as yet inconclusively demonstrated theory of Jiminez-Diaz (29) that failure of ammonia formation by the kidney is an essential renal defect in suprarenal insufficiency and leads secondarily to sodium loss. It is of interest, therefore, that total ammonia excretion in *J.X.* (fig. 2, table 3) bore no reciprocal relationship to sodium excretion after testosterone propionate, remaining, indeed, pretty much unaffected throughout. With the declining urine volume, the constancy of total ammonia excretion per collection period makes for an increase in the concentration of ammonia during the androgen effect but this increase falls far short of compensating for the diminution in concentration of sodium. The pH of the night urine studied (table 3) remained unaltered during the androgen effect, just as in Hartman's dog, and the $BHCO_3$ content and concentration of titratable acid were unchanged. Thus the relative proportions of acid and basic phosphates may be considered constant, while the total phosphates excreted per collection period declined, largely accounting for the decline in total titratable acid. During recovery there was a brief excess of base loss with a transient rise in pH and $BHCO_3$ and a fall in the concentration of titratable acid.

In the previous experiments (2) we had some difficulty in demonstrating the effect of testosterone propionate on creatine excretion in the eunuchoid because of the frequently inconspicuous and irregular spontaneous creatinurias of these people. The constant feeding of 1 gm. of creatine daily to *J.X.* provoked an intense and fairly constant creatinuria (fig. 1), so that the effect of testosterone propionate in inhibiting creatine excretion is well shown. The excretion of creatinine is never changed in our experiments. A slight rise in basal metabolic rate appeared in *J.X.* after testosterone propionate (fig. 1), at approximately the same time as in several of our previous patients, but the decline during recovery was unusually delayed. Blood pressure and pulse rate were unaffected (table 6).

The two normal men, *R.W.* (fig. 3) and *J.L.* (fig. 4), show responses to testosterone propionate which are in most respects similar in kind to those of the eunuchoids. The actual amount of nitrogen retained per day during the time of maximal effect of the androgen is somewhat less in *J.L.* and distinctly less in *R.W.* than in any eunuchoid studied except *H.S.*, whose hypopituitarism presumably interfered with the deposit of protein in his tissues (table 5). The amount of nitrogen stored per kg. per day, during the maximal effect of the androgen, 0.029 gm. for *R.W.*, 0.030 gm. for *J.L.*, is about half that for the eunuchoids without gross pituitary damage. There is no greater tendency for the retained nitrogen to be discharged more quickly during recovery than in the eunuchoid.

While the inorganic phosphorus in the urine declines in both normals, the changes in *R.W.* are much the more decisive, the decline in this instance being as great as that in the eunuchoid *J.X.* with either the 25 or 50 mg. dose. Urinary sodium and chloride excretion drop sharply with the start of treatment and then rise to a variable

degree and for a varying length of time until the influence of testosterone propionate has waned, when the urinary excretion of both of these electrolytes rapidly increases. In *J.L.* this rising sodium and chloride excretion during recovery does not exceed the baseline in the decisive manner characteristic of this phase in all of our other experiments in which the androgen produced definite effects. Too much should not be made of the irregular character of the excretion of these substances under the influence of the androgen in contrast to the smoother, steadier decline in the eunuchoid *J.X.*, with the 50 mg. daily dose, because in previous studies in eunuchoids (2), the depression of sodium and chloride excretion was not infrequently discontinuous. The spontaneous daily variations in the urinary excretion of sodium and chloride which are manifest during control periods certainly contribute to these irregularities. Because of these same variations, estimates of the degree of reduction in excretion of these electrolytes are inexact but, as far as our data go, no differences are evident between our normals and our eunuchoids (table 5). Depression in urinary potassium excretion by an average of 9 m. eq. per day is convincingly shown in R.W., with a subsequent resumption of baseline values after a brief supernormal phase during recovery. In *J.L.* irregularities of excretion blur the effect, but the same tendency is evident. The intensity of this influence on potassium excretion in these men is of about the same order as in the hypopituitary, *H.S.*, the eunuchoid *N.D.*, and the normal woman, *P.J.*, but considerably less than the 20 m.eq. reduction in excretion in the eunuchoid *J.X.*, with the 50 mg. daily dose of testosterone propionate. The gains in weight for these normal men after 8 days of treatment, R.W., 0.8 kg., *J.L.*, 1.6 kg., fit well with those of the eunuchoids, which range from 1.0 to 2.6 kg. per 10-day period of treatment. No alteration in B.M.R., fasting respiratory quotient, blood pressure or pulse rate was produced by testosterone propionate in the amounts given (table 6).

As previously mentioned, the concentrations of sodium, potassium and chloride in the serum were unchanged during the course of the testosterone propionate effect, while that of inorganic phosphorus declined slightly to rise during recovery (table 4). Non-protein nitrogen of the whole blood in R.W., however, and urea nitrogen of the whole blood in *J.L.* showed the same tendencies although with certain irregularities, toward lower values during the later phases of the testosterone propionate effect, with increases during recovery, that were apparent in the eunuchoids, *J.X.*, (table 2) and *N.D.* (2).

Before testosterone propionate injections, R.W. was excreting an average of 83 I.U. of androgenic activity per day for 8 days, and *J.L.* an average of 78 U per day for 6 days, both values being well up in the normal range (26, 30, 31). The natural androgens of these normal young men, however, are not exerting metabolic influences to the fullest extent to which the organism can respond. Indeed the effects of added testosterone do not differ in kind from those obtained in the eunuchoid, and distinctions in degree can thus far be made only with regard to the nitrogen retaining property. It is of interest that Thorn and Engel's impotent, but otherwise normal man (14) retained 0.022 gm. of nitrogen per kg. per day, not far from the 0.030 of our normals at a comparable time in the experiment. Our normal young men responded to testosterone propionate in a manner similar to Thorn and Engel's normal dog (14) and unlike the negative experience of Kochakian and Murlin (12) with androstenedione in their normal dog. Normal men of riper age, however may conceivably have a more intense secretion of testis hormone than our 20-year-olds and the effects of added androgens may be less accordingly. This remains to be determined. Age itself, between the years of 27 and 50, would appear to have little modifying influence on

these reactions, as the eunuchoid *M.D.*, who was 51, responded as well to the testosterone propionate as did younger eunuchoids.

The two normal women contrast strongly with one another in their response to testosterone propionate. The general course of the curve of nitrogen excretion in *P.J.* (fig. 5) was similar to those in the normal and hypogonad men. The average daily reduction in urinary nitrogen excretion during the time of maximal effect was 2.61 gm. (table 5), practically identical with that in the normal man *J.L.*, and only about 20% less than that of most of the eunuchoids. The 0.043 gm. of nitrogen estimated as retained per kg. per day fell between the 0.030 gm. of the normal men and the 0.060 gm. of the average eunuchoid without gross pituitary disease. *E.S.* (fig. 6), the second normal woman, on the other hand, showed a doubtful nitrogen retention, amounting to only 0.73 gm. per day during the time of maximal effect or 0.016 gm. per kg. per day, by far the lowest values in our series. The curve of inorganic phosphorus excretion in *P.J.* was much the same as in the normal man *R.W.*, or in the eunuchoid *J.X.*, and the degree of depression in urinary excretion was of the same order (table 5). This urinary constituent of all those studied in *E.S.* showed the most convincing evidences of a testosterone propionate effect. The average daily reduction in excretion of 0.090 gm. in *E.S.* was less than the corresponding 0.150 gm. in *P.J.* but this constituted a decline of 25% for *E.S.* as compared to one of 20% for *P.J.*

The decline in sodium and chloride excretion in *P.J.* under the influence of the androgen was slight and defined chiefly by the sharp diuresis of these constituents during recovery. No evidences of their retention were seen in *E.S.* Urinary potassium excretion declined somewhat in *P.J.*, and the constant pretreatment baseline, together with the restitution of these baseline values at the end of the experiment attests to the genuineness of this effect. The changes in *E.S.* are far too slight to have meaning. The gains in weight again show contrast between the 2 women. *P.J.* gained 1.6 kg. in characteristic pattern and was losing weight at the termination of the experiment, while the increase of 1.3 kg. in *E.S.* reached its peak by the 6th day of treatment, dropped off somewhat before the injections were stopped and remained, largely as a net gain of 1.00 kg., at the end of the experiment. Creatinuria seems to have been inhibited in *P.J.* by testosterone propionate, with a return towards the baseline during recovery. The changes in creatine excretion in *E.S.* are suggestive of the same influence in her, but the absence of the distinctive rise during recovery, together with the uncertainties surrounding the measurement of such small amounts of creatine as are involved, makes the experiment inconclusive. In neither patient were there significant alterations in oxygen consumption, fasting respiratory quotients, pulse rate, or blood pressure following testosterone propionate (table 6). Calculations of the non-protein respiratory quotient in *P.J.* showed a slight increase in carbohydrate and fat oxidized at the expense of protein, much as in the eunuchoid *F.R.* (2).

Thus, in the normal woman, the characteristic metabolic effects of testosterone propionate may appear in well defined form as in *P.J.*, or be slight, partial and often inconclusive as in *E.S.* The reason for the differences is not clear. The mature, menstruating woman is a difficult subject for metabolic experiments dealing with salt and water excretion. Spontaneous gains in weight, largely due to water retention are frequent just before menstruation or at the mid-interval and on rare occasions may result in gross edema (16, 32, 33). Thorn, Nelson and Thorn (16) observed declines in urinary sodium and chloride excretion and in urine volume to coexist with the weight gains in several subjects on constant food and fluid intakes, and attributed these phenomena to the salt retaining properties of the steroid ovarian hormones. Androgen excretion has certainly never been shown to vary in any periodic fashion

during the cycle (26, 35), and possible changes in the secretion of the adrenal cortical 'salt and water hormone' must remain a mystery for the present, so that the view of Thorn and his coworkers is entirely reasonable.

The effects of testosterone propionate in our subjects, therefore, may thus be superimposed upon those changes in sodium, chloride and water excretion that are likely to be proceeding in the body under the influence of its own hormones, and sharp distinctions between the influence of extrinsic and intrinsic hormones becomes difficult to draw. Neither of our 2 women, however, showed any spontaneous gain in weight immediately before the menstrual flow preceding our collection period, nor any loss of weight during or after the bleeding, so that a presumption exists against the weight gain in *P.J.* being a natural process. Furthermore by virtue of the unexpectedly early bleeding occurring in *P.J.* at 23 days, 4 days after stopping testosterone propionate, rather than at her usual 28 to 30 days, it is rather doubtful whether the premenstrual phase covered by the experiment was physiological or whether it represented merely a period of androgen influence preceding a spurious menses induced by testosterone withdrawal.

Nitrogen and phosphorus excretion show no periodic variations in the careful balance studies of Sherman, Gillett and Pope (32) on 3 cycles in 2 normal women, save for a drop in nitrogen excretion in the urine, the first day of menstruation, nor do Thorn and his coworkers (16) indicate any such variations in their subjects. Urinary potassium excretion appears from Thorn's work to be less strikingly involved in periodic electrolyte changes than do sodium and chloride excretion, although in one subject charted a slight increase appeared premenstrually. It seems legitimate, therefore, to conclude that the apparent effects of testosterone propionate on the urinary excretion of nitrogen, inorganic phosphorus and potassium, which were so conspicuous in *P.J.*, were real and not due to concurrent variations in the secretion of her own hormones. Similarly, the failure of *E.S.* to respond so clearly to testosterone propionate probably cannot be attributed to interferences with the experiment by her own endocrine secretions.

The slighter response of *E.S.* to the injected testosterone propionate cannot be explained by the theory that her organism was already under a greater load of natural androgens than was that of *P.J.* if we measure this load by the androgenic activity of the urine. Thus *P.J.*, who was excreting an average of 100 I.U. of androgenic activity per day for 8 days reacted well to injected testosterone propionate, while *E.S.*, who was excreting 64 U per day for 8 days reacted less decisively. More comprehensible correlations between natural androgen load and response to added androgens may become apparent, however, when our technics of urine assay provide for measurement of quantities of substances rather than total activities of mixtures of substances, and when the nature of the physiological androgens of the female and the course of their metabolic transformation into urinary representatives becomes better understood.

The nature of the changes in the tissues of the body induced by testosterone propionate can only be indicated in a rough and approximate way by such studies as ours, but enough has been learned to arouse our interest in them and our belief in their importance. It must be kept in mind that all of the effects discussed, with the exception of those on the male genitalia and their accessories may be indirect and involve the participation of other glands of the body to an as yet undisclosed degree. Testosterone propionate as far as it has now been studied in man and animals with intact adrenals, is adrenomimetic only in regard to diminishing urinary sodium, chloride and water excretion. Even in this function, the absence of a reciprocal rise in ammonia excretion suggests differences from the action of the adrenal cortical

hormone. Inorganic phosphorus and nitrogen excretion are not diminished by the adrenal hormones as they are by testosterone propionate, and potassium excretion is increased rather than reduced.

The amounts of nitrogen, calculated as protein, estimated as retained, from the beginning of treatment to the onset of the recovery period, came to from 102.7 gm. in 12 days for the normal man, R.W. to 252 gm. in 15 days for the eunuchoid J.X. (second experiment), all far in excess of any conceivable requirement of growing prostate and seminal vesicles, the changes in which even in the eunuchoid are barely detectable in such brief experiments. In the normal woman P.J. 117.9 gm. of protein were retained in 9 days. While Korenchevsky and Hall (36) have pointed out that protracted treatment of female rats with testosterone propionate will cause massive enlargement of the uterus and vagina, such was not the case in Zuckerman's experiments in the monkey (37), with doses much like our own; nor does it seem likely that any such 10-fold increase in the size of the uterus required to account for the protein deposit in P.J. could have occurred in the brief period of our experiment. We are thus left with the probability that non-genital tissues of affected normal women hold the important portion of retained nitrogen as do those of men.

We have already commented on the considerable reduction in the concentrations of blood urea nitrogen and N.P.N. that are observed toward the later phases of the testosterone propionate effect. These changes, which are demonstrably not due to blood dilution, are reminiscent of the depression in the concentration of these substances in the blood of the dog under the influence of the growth hormone from the anterior lobe of the pituitary body, as described by Teel and Watkins (38). During recovery the concentrations of these affected constituents again rise, the entire process being compatible with a retention of nitrogen in the tissues themselves during the androgen effect and release thereafter. Even if all of the 2 kg. weight gain induced by testosterone propionate in J.X. (second experiment) be assumed to be extracellular fluid, the nitrogen estimated as retained by virtue of expansion of this compartment amounts to not more than 5 gm. out of a total of 42 gm. for the period under discussion. Expansion of the volume of whole blood would, of course, make the amounts of circulating protein retained a more important fraction of the total, so that this point requires experimental study. Kochakian and Murlin's studies in the dog (11) indicate no unusual fecal nitrogen losses, accompanying the reduction in urinary nitrogen excretion.

The association of reduction in the urinary excretion of potassium and inorganic phosphorus with that of nitrogen is in accord with the conception of an augmentation of protoplasmic mass under the influence of testosterone propionate. However, we cannot quite yield to the temptation to speak of this as retention in the absence of studies of fecal excretion. The continued reduction in urinary potassium excretion of 7 to 20 m. eq. per day, in our experiments, nevertheless, cannot be accounted for by the increase in extracellular fluid or by a reasonable increase in volume of blood containing the usual 50 m. eq. of potassium per liter, so that, unless there is a compensatory fecal loss, potassium must be retained within cells. Similarly the continued reduction in urinary excretion of inorganic phosphorus of 90 to 190 mg. per day cannot be attributed to increase in extracellular fluid or to hypothetical expansion of the volume of blood, containing 390 mg. of total phosphorus per liter, so that here too cellular deposit must be invoked, unless a compensatory fecal loss can be demonstrated. When complete balance studies are available ratios of retained potassium to nitrogen and phosphorus to nitrogen will provide interesting information concerning the influence of androgens upon tissues. It is of especial importance in this connection that the recent careful balance studies of Sandberg, Perla and Holly (39) have dis-

closed reduction in the retention of nitrogen, sulfur, sodium, potassium and chloride after castration in the rapidly growing 7-week old rat. No such effect however appeared after castration of mature animals.

The evidences which we have adduced for a somatotropic influence of the androgens in man, although incomplete in certain respects, provide a needed hypothesis for the interpretation of the striking if limited body growth and considerable muscular development that may accompany precocious puberty in man. Studies in animals which will be necessary for the more precise definition of these somatotropic effects have just begun to appear. Thus Papanicolaou and Falk (40) have reported that castration reduces the size of the temporal muscles of the male guinea pig, and that testosterone propionate causes hypertrophy of these muscles in the castrated immature male and in the spayed and normal adult female. The general muscle mass also increased. Korenchevsky, Dennison and Brovsin (41) have reported that castration reduces the size of the heart, liver and kidneys of the male rat and that testosterone propionate restores the weights of these organs to normal. More extensive data of this kind is greatly needed. Thus far no really considerable growth of the body as a whole has been produced in animals by androgens.

SUMMARY

In confirmation of our previous studies, intramuscular injections of 25 mg. of testosterone propionate, daily or twice daily, in a eunuchoid, produced reduction in the urinary excretion of nitrogen, sodium, potassium and chloride, together with a gain in weight, due largely to water held in association with the salts and protein retained. A considerable reduction in the urinary excretion of inorganic phosphorus accompanied that of the other constituents. During recovery sodium, chloride and water were rapidly lost from the body, inorganic phosphorus and potassium less rapidly and less completely, while nitrogen stored was retained in great part for weeks.

Two normal young men responded like the eunuchoids, except that the 0.03 gm. of nitrogen retained per kg. per day, during the period of maximal effect, was half that of the eunuchoids.

One normal young woman responded essentially as the eunuchoids and the normal men. The second normal young woman showed a definite reduction in the urinary excretion of inorganic phosphorus but otherwise distinctly less evidence of a testosterone propionate effect. The reasons for the differences between these women are not clear.

The concentrations of urea and non-protein nitrogen of the blood were definitely reduced by such treatment, while those of hemoglobin, plasma proteins, and serum sodium, potassium and chloride were unaffected. Inorganic phosphorus concentration in the serum was doubtfully reduced.

The reductions in urinary electrolyte excretion, studied in the eunuchoid, were unaccompanied by important changes in urinary ammonia, pH, bicarbonate or concentration of titratable acid.

Creatine excretion, in the eunuchoid, intensified by ingestion of creatine, was substantially reduced by the androgen and increased during recovery.

Percutaneous administration of 25 and 50 mg. daily of testosterone itself in an ointment, in the eunuchoid, produced diminution in the urinary excretion of nitrogen.

The basal metabolic rate, fasting respiratory quotient, pulse rate and blood pressure of the normal subjects were not conspicuously affected by testosterone propionate. The basal metabolic rate of the eunuchoid was probably slightly increased; the pulse rate and blood pressure were unaffected.

The 102.7 to 252.2 gm. of protein estimated as retained by these subjects is not accounted for by increases in the bulk of genital tissues and represents deposit of new material elsewhere in the body. This together with the reduction in the urinary excretion of inorganic phosphorus, potassium and creatine points to a somatotropic influence of androgens, and provides a clue to the explanation of unusual body growth accompanying precocious puberty.

We wish to acknowledge our obligation to the several technicians of the Clinical Chemistry, Basal Metabolism and Metabolism Clinic laboratories of the Department of Medicine and to Miss Mildred Neevel and her nurses of the Metabolism Unit of Albert Merritt Billings Memorial Hospital for their loyal labors, so necessary for our work. We wish furthermore to express our gratitude to our patient J.X., and to our volunteer normal subjects, Misses Pauline Jones and Eugenie Schweitzer and Messrs. Robert Williamson and Jerome Lerner.

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OBSERVATIONS ON THE CONTINUED USE OF MALE SEX HORMONE OVER LONG PERIODS OF TIME

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WE HAVE observed the immediate effects in a number of patients. In the cases of hypogonadism in males, there has been, almost universally, a growth of the penis and scrotum, increase in pubic hair, increase in the number, frequency and degree of penile erections, and increase in libido. These observations have been reported elsewhere and by others. But the questions which have arisen, in the minds of the patients, the patients' family and the physicians have been: (*a*), what does the future hold; (*b*), will the same effect continue or decline, or perhaps (*c*), will the patient ultimately be able to dispense with the therapy, (*d*), will the immediate dose have to be continued, increased or decreased, (*e*), will the continued use affect other glands such as the pituitary, and create other problems, (*f*), what changes can one hope for from long continued use, that may not have been observed over the shorter periods. With these problems and questions in mind this brief clinical report is given.

Case 1. M.F. first came under observation in 1935, at the age of 26 years. His family history and past history were unimportant, excepting the fact that he had mumps at the age of 10, but there is no information or suggestion that an orchitis occurred.

Until the age of 13 years, he considered himself a perfectly normal boy (the manifestations of his condition may have been present, but were unobserved). He was thin, very active, alert and athletic. His first insight into his difficuly appeared at school when he realized that his classmates were developing adult-like sex characteristics, while his remained unchanged. Because of these deficiencies, he was teased and ridiculed endlessly and as a result used much ingenuity to keep his secret undisclosed. But he began gaining weight excessively and his breasts became large and pendulous. This tormented state increased until he entertained thoughts of suicide.

On admission he complained chiefly of the following: infantile genitalia; complete lack of sex desire, sex urge, libido and erections; obesity; large and pendulous breasts; high pitched voice; complete absence of facial and axillary hair and exceedingly scant pubic hair; female mannerisms, movements and carriage; extreme shyness.

His initial physical examination showed a tall, obese, male adult (weight 215 lb.) with a high pitched voice, immature beardless face, only slight pubic hair, no axillary hair, infantile penis, testes and scrotum, and large breasts. His blood pressure was systolic 155, diastolic 75. His measurements were as follows: total height 72½ in., symphysis pubis to floor 37½ in., symphysis pubis to top of head 35 in. and span 74½ in. B.M.R. was 10% above the average. The sugar tolerance curve was as follows: blood sugar 123 mg. before glucose; 1 hour after the administration of glucose (1.75 gm. per kg. of body weight) 219 and 134 mg. at the end of 2 hours. X-ray of the skull showed a sella turcica 50% smaller than the average normal. X-ray studies of the renal and suprarenal regions, including pyelograms (with dye given intravenously and by retrograde injection), showed no indication of abnormal pathology. Visual fields showed no departure from the normal, excepting a slight constriction to

colors. This was not considered of pathological significance. The results of urinalysis, blood count and blood chemistry studies were normal. The result of a Wassermann test was negative.

We regarded the patient as suffering from a pluriglandular dysfunction with hypogonadism and eunuchoidism as the chief problems. The sugar curve and blood pressure were unusual for the usual anterior-hypopituitarism. There was no direct evidence of suprarenal disease. Treatment at first consisted of gonadotropic substances by injections, 100 injections of follutein (Squibb) and antuitrin-S (Parke, Davis & Co.) in doses of 200 R.U. given about 3 times a week. He was placed on a low caloric diet. At the end of a year, he showed very few slight changes; he had lost 30 lb. and there was a very slight increase in pubic and axillary hair. It was then decided to perform a bilateral mastectomy, for cosmetic reasons and to allay his mental anguish created by the presence of the large breasts. This was successfully performed, but his major problems remained the same and in a few months he had regained his lost weight. There were still no signs of change in genitalia or sexual feeling.

On October 1, 1937, treatment with testosterone propionate[1] was started, 1 mg. every other day for 15 injections. There was no response. Then 25 mg. was administered every second day. Within 2 weeks a dramatic response had occurred. The penis and scrotum began enlarging rapidly, then reddened, frequent, numerous erections occurred and the friction of the clothes led to onanism, with distinct orgasm. Soon ejaculation of a few drops of a clear albuminous material occurred, later this increased to about 2 to 3 cc. Hair growth (suprapubic and axillary) progressed, scrotal rugae became evident and some facial hair appeared. The voice gradually assumed a lower pitch, the pomum adami became prominent and his female characteristics and carriage disappeared. The dosage was reduced to 25 mg. twice a week and then to 10 mg. twice a week. It soon became evident that the previous gains were disappearing. He was put back on 25 mg. every third day. At present he has a distinctly masculine carriage and voice, he attempts facial shaving several times a month, erections occur frequently, the penis being about 4 in. in length (erect) and 3½ in. in circumference (originally it was impossible to obtain any measurements because of its shrunken and soft structure). The scrotum is adult in texture and appearance, the testes larger and firmer. He has attained self-assurance, lost his shyness and his libido has been considerably increased.

Discussion. The diagnosis is open to a great deal of discussion and at best is only conjecture. Still, the presence of the hypogonadism and eunuchoidism was predominant. There was minimal response to gonadotropic substances. The response to testosterone propionate was definite, continued and dramatic. On several occasions he has been kept off the substance for a week or two. Within about 5 days recession had commenced and an apparent reversal had set in. Once at the end of 2 weeks he seemed to lose half or more of his gains. As striking as the physical and objective gains are the subjective and mental changes; he is now definitely 'masculine.' He has received about 2000 mg. in all and it seems that the minimal maintenance dose is about 50 to 60 mg. a week. He has been injecting himself regularly for the past 8 months without any difficulty or complications. His attitude is comparable to that of the patient with diabetes mellitus who injects himself with insulin. We have observed no ill effects and feel that his future is much more than could have been hoped for in such a case prior to 1936. His only concern is that the substance will be available for life. Within the past month examination of the ejaculate revealed a few prostatic bodies and a few immature non-motile spermatozoa.

Case 2. R.R. first appeared at our clinic in 1931, at the age of 16 years. His family history was irrelevant. Chicken pox, pneumonia, diphtheria and chorea occurred during infancy and childhood with no known sequelae. He was known to have bilaterally undescended testes and congenital inguinal herniae. At the age of 8 years, operation was performed to correct

[1] The testosterone propionate used in all our cases was oreton, supplied by Schering Corporation, Bloomfield, New Jersey, through the kindness and courtesy of Dr. Max Gilbert.

the herniae and an attempt was made to 'pull' the testes into the scrotum and 'fix' them there. On the left side, a small testicle was found, but no definite testicular tissue was found on the right side. The operative wounds sloughed and some months later a second operative intervention was considered necessary. At the age of 10 or 11 he was observed to be 'growing rapidly.' At 14 years of age an appendectomy was performed. His growth continued; he observed that his shape was assuming a distinctly feminine contour and that his legs and arms were exceedingly long. The pitch of his voice grew higher. His genitalia remained small and shrunken; there appeared to be no scrotal tissue aside from a slight suggestion. No facial hair, practically no axillary hair and no pubic hair appeared. There were no sexual urge feelings, erections, nor even the slightest interest. Rather, he was despondent, 'blue' and entertained suicidal thoughts.

His physical examination, at 16 years of age showed a tall, well-developed young man, with prominent female-type hips and disproportionately long, well-shaped legs and thighs. Weight was 147 lb. His height was 68½ in., span 68½ in., lower measurement from symphysis pubis down was 35½ in., upper measurement was 33 in. Results of an examination of head, neck, heart and lungs were negative. The abdomen showed no abnormalities. There were bilateral herniorraphy scars (depressed and irregular). The scrotum was negligible, thin, small and contained nothing. The testes could not be palpated anywhere. The penis was ¾ in. long (at the most) and less than pencil diameter. There were a few scant fine pubic hairs. The patient's carriage, shape, contour, voice, etc. was feminine.

His blood pressure was 120/70. His B.M.R. was −20%. Sugar tolerance curve was regarded as normal or slightly increased. Findings of urinalysis and blood count were normal. The result of a Wassermann test was negative. X-ray of the skull showed a relatively small sella turcica (about 80% normal size). The visual fields were normal.

Therapy from 1931 to 1937 (when he was 22 years old) consisted of thyroid ext. (U.S.P.) from 3 to 6 grains daily, but his B.M.R. remained between −10% and −20%. He also received numerous courses of injections of gonadotropic and APL substances. During this period from 1931 to 1937, the penis grew to about 1½ in. in length, occasional erections began to appear, a few more pubic hairs appeared and attempts at masturbation were made. He continued to grow, his height now being 73½ in., his lower measurement being 39 in., his upper measurement 34½ in. During 1937, he became more acutely aware of his condition and his sexual shortcomings. He became depressed, despaired of his opportunities for love, sex life and marriage and cried frequently.

In November 1937, testosterone propionate, in 10 mg. doses was administered every third day. In about 3 weeks, distinct and dramatic changes began to take place. The penis enlarged to 2½ in. when erect and erections occurred very frequently. The dose was now increased to 25 mg. 3 times a week. At once, further growth of the penis and scrotal tissue was marked. The erections were more frequent and marked libido appeared. Masturbation led to orgasm with emissions and ejaculation. The growth of hair, pubic and axillary, on the extremities and face progressed. He began to shave once a month.

He has continued the injections himself; 25 mg. every second, third, or fourth day. He became energetic and entered upon business ventures. His penis now measures about 3 in. when at rest and flaccid, and almost 5 in. when erect, with a circumference of about 4 in. Ejaculation is now about 2 cc. (no spermatozoa). His voice is deep, his carriage and movements distinctly masculine. He has had a total of about 2600 mg. of testosterone propionate to date. Coitus is performed regularly and satisfactorily.

Discussion. This patient was apparently a normal child (?) but had undescended testes. Sloughing of the testes following an operation, produced a castrate and he grew into a typical eunuch. Treatment with gonadotropic substances was instituted in the hope that any testicular tissue present, as well as other genital tissue, might respond. There was some response as described. However, in spite of the response, much more was desired. The response to testosterone has continued to improve his state, mental and physical, until his sexual feelings are normal and the size of the penis normal. He injects himself without difficulty regularly, and regulates his dose

which averages about 50 to 75 mg. per week. There have been no untoward symptoms or signs and omission of injections is followed after 5 to 7 days by a lessening of the erections and a shrinkage of the penis. He is happy, content, hopeful, has a college education and is entering business. He contemplates marriage and we are to explain to his fiancee that he is deficient in testosterone, similar to the diabetic's deficiency in insulin.

Case 3. H.W. appeared in March 1937, at the age of 21 years, with the following history. At the age of 3 years, he had bilateral otitis media and pneumonia. Between the ages of 5 and 6 years, he had measles and chicken pox. He appeared to be normal, although slightly smaller than average, until he was about 13 years of age when it was observed he was not

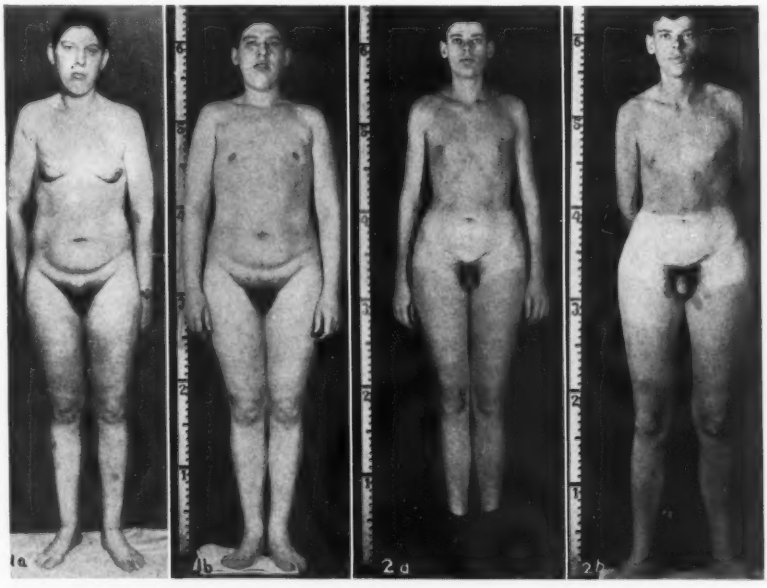

Fig. 1a. Case 1. *M.F.*, Just prior to operation (mastectomy). Fig. 1b. Case 1. *M.F.*, 6 months after commencing treatment with testosterone propionate. Fig. 2a. Case 2. *R.R.*, Just prior to commencing treatment with testosterone propionate. Fig. 2b. Case 2. *R.R.*, 6 months after commencing treatment with testosterone propionate.

developing genitally in proportion to his age and that his testes were undescended. This lack of genital development became more manifest at 14 and 15 years of age and he was advised to have an operation to place the testes into the scrotum. This was done in 1932, when he was 16 years old. The operation was only partly successful, as the testes became fixed at the upper end of the scrotum. In 1935, when he was 19 years of age, he looked like the average boy of 13 or 14 in height, shape, stature, facial expression and sexual development. From June 1936 to March 1937, he received injections of gonadotropic substances, once or twice weekly, but his improvement was very slight. When he presented himself in March 1937, at the age of 21 years, his complaints were as follows: lack of growth for the past few years (his height was 63½ in.); complete lack of facial, axillary, body and extremity hair; pubic hair very scant, short and immature; genitalia exceedingly small (about 1½ in. in length, maximum); rarely occurring erections; complete lack of libido; childish facies and mannerisms and a child-like, high-pitched voice. He had completed high school, had had about 2½

years of college and 1½ years of business school training, but because of his stature, appearance and mannerisms, he had been unable to accomplish anything, or obtain adult employment.

Physical examination substantiated the complaints made. One would guess his age to be about 14 or 15 years. There were no signs of facial or body hair except a few pubic hairs. Penis, scrotum and testes were very small and almost infantile. The testes were at the upper end of the scrotum and about ½ in. to ¾ in. long. His weight was 110 lb. and had been 120 lb. the previous year. A sugar tolerance test showed a flat curve (90, 96, 90 mg. sugar per

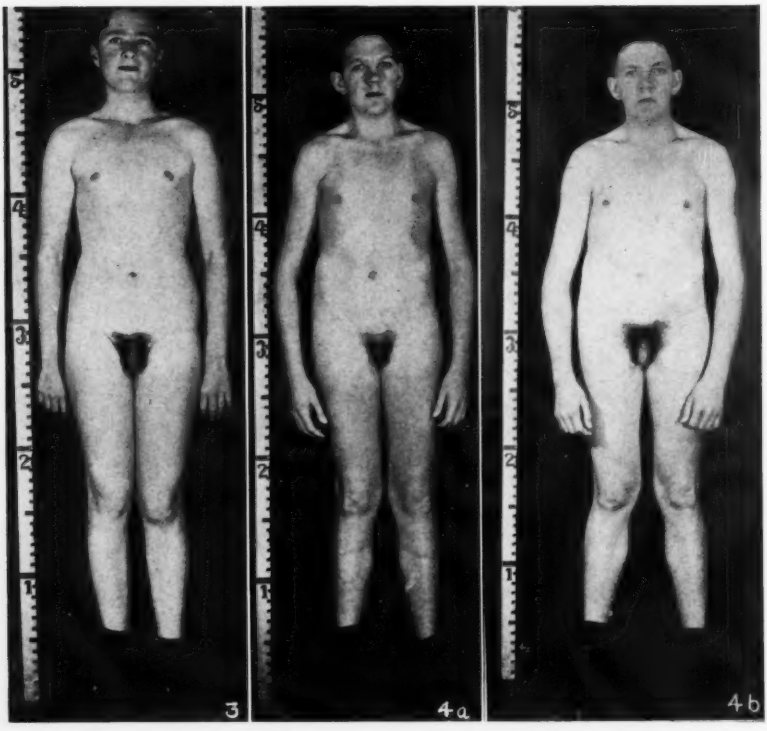

Fig. 3. Case 3. *H.W.*, After 3 months treatment with testosterone propionate. Fig. 4a. Case 4. *O.R.*, Just prior to commencing treatment with testosterone propionate. Fig. 4b. Case 4. *O.R.*, 6 months after commencing treatment with testosterone propionate.

100 cc. blood). The B.M.R. was −14%. The sella turcica was somewhat smaller than average. Blood pressure was 100/65. Height was 63½ in., lower half 31 in., upper half 32½ in., span 62½ in.

He received 30 injections of follutein (500 u each) and 30 injections (Squibb) anterior pituitary alkaline extract (2 cc.) in 10 weeks. He then received 30 injections of growth hormone (Ayerst, McKenna & Harrison) of 2 cc. each and 30 injections of A.P.L. (Ayerst, McKenna & Harrison) of 1000 u each. At this time his height was 64½ in. and a few more pubic and some axillary hairs were observed. There was but slight growth of the genitalia. He then received 30 injections of G.A.P. (Ayerst, McKenna & Harrison, a preparation containing growth hormone, adrenotropic hormone and prolactin), one cc. at each injection and 30 injections of anterior pituitary gland sex factor (maturity factor of Ayerst, McKenna &

Harrison), 2 cc. each injection. These 180 injections had been given over a period of about 30 weeks. Some improvement, it is true, was evident. In November 1937, injections of testosterone propionate, 20 mg. 3 times a week were commenced. Within 3 weeks, the changes were striking and continued to increase. By May 1938, his erections were numerous and on several occasions episodes of priapism resulted. The penis, testes and scrotum had enlarged; the penis in erection now measured about 5 in., the testes were completely down in the scrotum and about 1½ in. in length. The scrotal rugae were marked and well formed. Pubic and axillary hair became abundant. The voice became adult and low pitched. The pomum adami became marked. Facial hair began to appear. Masturbation, which at first resulted in no orgasm now yielded orgasm with 2 to 4 cc. of semen (several immature spermatozoa were observed in June 1938). Carriage and behavior became more adult in nature. In July, he began to perform coitus (and in 3 days cohabited 4 times with satisfactory results). During July he received 10 mg. on only 4 occasions and in August received no testosterone propionate, without any apparent recession or diminution in the degree of libido and erections. His height is now 65¼ in.

Discussion. The diagnosis of a modified Lorain-Levi syndrome of anterior hypopituitarism was made. When he first appeared, he had already been operated on for undescended testes and had had some treatment (gonadotropic substances). Further use of growth hormones and gonadotropic substances produced some slight further improvement, but the outstanding and most striking results followed the use of testosterone propionate. He received in all about 2100 mg. In July he received very little testosterone propionate and in August none, but the expected regression failed to materialize. We are hardly prepared to believe that he may now carry on without requiring any further treatment, but if the state of his pituitary and testis function have been improved, it is not inconceivable that he may progress without therapy. At any rate, from our experience we have the feeling that if necessary, we can resort to treatment again when and if indicated.[2]

Case 4. O.R. first appeared, at the age of 24 years, in December 1933. His family history is of no importance and his previous medical history includes measles, chicken pox and pneumonia during early childhood. He had graduated from high school at the age of 17 years and has worked as a clerk since. He stated that since infancy or childhood, he and his parents have known that his genitalia were small and infantile and had not grown at all. Physicians who had been consulted advised him to permit 'nature to take its course,' and that treatment would be of no avail. His complaints were: infantile genitalia; absence of a 'real scrotum'; absence of facial and axillary hair and very scant pubic hair; complete lack of libido and erections and a high-pitched voice.

Physical examination showed a male adult, appearing much younger than his stated age of 24 years. There was no facial, body or axillary hair and but few pubic hairs. The penis was about ¾ in. in length and infantile. No real scrotal tissue was present. No testes could be found. Physical examination was otherwise normal. Blood pressure was 110/70. B.M.R. was −8%. Sugar tolerance test results were as follows: 86, 92, 90, mg. per 100 cc. of blood. X-ray of the sella turcica showed it to be very small (about one-third smaller than normal). His weight was 108 lb.

From January 1934 to October 1935, he received injections of follutein (500 u) and alkaline pituitary extract (Squibb) 2 cc., 3 times each week with very little change. From October 1935 to October 1936, he received maturity factor (Ayerst, McKenna & Harrison) 1 to 2 cc., and A.P.L. (Ayerst, McKenna & Harrison) 1000 u, each, 3 times a week. A little further improvement in hair growth (axillary and pubic) was noted. Occasional erections occurred; the penis grew to about 1 in. to 1½ in. No further changes were evident. In October 1936, testosterone propionate injections were begun. At first, he received 2.5 mg. every other day, then 5 mg. every other day. Some changes began to be manifest in about 6 weeks. Dis-

[2] Since writing, he has resumed treatment (50 to 75 mg. per week) as he is convinced he is better in every way while under treatment. He has now received 2800 mg. in all.

couraged by the failure, all treatment was discontinued until October 1937, when treatment was resumed, 25 mg. every other day. In 2 or 3 weeks the changes described in the other cases became evident. The largest measurement of the penis, however, was about 2¾ in. in length and 2 in. in circumference (erect). Libido increased, and a general feeling of strength and well being supervened. His weight rose rapidly from 110 lb. to 130 lb. He became masculine, aggressive and alert. However, in May 1936, he tired of the injections (he was injecting himself) and ceased. Gradually all the gain began to subside. In August he returned and requested that he again be given treatment and again is obtaining the desired results.

Discussion. This patient undoubtedly was the severest case of the group, with an infantalism due to anterior pituitary deficiency. All therapy prior to the administration of testosterone propionate gave but little improvement. The testosterone propionate produced striking results. However, being 29 years of age, it seems that the time for optimum response had passed; the deficiency of years could not be effaced and only partial response resulted. In May 1938, he again despaired and thus omitted treatment until he realized that the improvement obtained from October 1937 to May 1938, was valuable and beneficial, not only 'genitally,' but in general well being, strength, better mental state, and happiness. He had used 1800 mg. from October 1937 to May 1938, and during August 1938 resumed self-administration, again with startling response, making the total now 2700 mg.

SUMMARY AND DISCUSSION

In no case have any ill effects or untoward symptoms or signs appeared. On several occasions the erections have been too numerous and libido too pronounced. However, decreasing the dose or frequency of injection soon overcame this. Symptoms and signs of masculinity have been proportionately improved. Improvement in general well being and strength was most marked. The mental response and improvement in outlook was excellent.

It is our impression from the use of about 20,000 mg. that as yet no real dangers have made their appearance. Several patients have been administering the material to themselves for about one year. It appears that the average maintenance dose may be between 50 and 75 mg. per week but sometimes more or less. The required dose is as yet open to discussion and apparently will vary in each case, just as there is no fixed dose of insulin. The simpler cases, such as castrates or eunuchs, who were more or less normal originally, will respond better than other cases, quicker and more satisfactorily, especially if treatment is not delayed too long after the pathological condition appears. The pituitary cases (hypopituitarism, infantalism, Lorain-Levi syndrome, Frölich's syndrome, etc.) will respond better than the older patients, 20 to 30 year old or older. (This report does not deal with adult impotency, sexual neurosis, hypertrophied prostate, senile sexual decline, homosexualism, senile debility, or use in the female.)

It seems that occasionally treatment may be discontinued without regression. Usually treatment must be continued, just as with other endocrine replacement therapy. We propose to teach each patient to administer his own injections after attaining maintenance dosage. It appears to us that the sale of the substance may have to be restricted to physicians, or on prescription only. The potential dangers of failure to do so become apparent to all interested in the problem.

In 2 cases, *R.R.* and *H.W.* there was distinct increase in prostatic size, from a non-palpable prostate, to a normal, or slightly less than normal size. In *M.F.* the prostate, which was non-palpable originally, became only suggestively enlarged, while in *O.R.* no prostatic increase could be made out at any time. *H.W.*, *R.R.*, and *O.R.* gained weight, there being no restriction on food intake or activities. *M.F.* was

kept on a low caloric diet and encouraged to lose weight, which amounted to about 25 lb. There was some rise in B.M.R. in each case but, what was more interesting, was that whereas thyroid administration had little, or comparatively little, effect early in their history, its effect on the B.M.R. was more marked during the periods of administration of testosterone propionate.

Only one case showed further descent of the testes (*H.W.*) while no testes could be found at any time in *O.R.* and *R.R.* In both *H.W.* and *M.F.* the testes became larger and firmer. There is apparently no danger of creating an abnormally large penis, although definite enlargement occurred in all, varying extremely in degree, most in *R.R.* and *H.W.* and least in *O.R.* Some slight edema of the penis and scrotum was present for a short time at the onset of treatment but disappeared soon. No other edema was observed.

ADDENDUM

SUBCUTANEOUS IMPLANTATION OF TESTOSTERONE TABLETS IN HUMANS

Following the suggestions of Deanesly and Parkes, et al., who reported on the implantation of testosterone tablets in animals, several of the cases described above (*M.F.*, *R.R.* and *O.R.*) and a number of others, were subjected to subcutaneous implantation of pure testosterone tablets. In each case, injections of testosterone propionate had been continued for months (or up to 2 years), to observe and obtain the true results of injections as described above. Tablets of pure testosterone[3] (compressed and sterile) each weighing 200 mg. were used. Under local anesthesia, an incision, about one inch in length, was made in the left posterior-axillary line, the skin undermined, the tablet inserted in the pocket thus formed, and skin sutured. No pain, nor inconvenience, whatsoever, followed. The full and dramatic effects of the testosterone propionate, as evidenced by the previous injections, were now maintained without injections. The subsequent course of events, duration of effects, etc., are to be described in detail in a further communication. At the end of 8 to 10 weeks, the effects of testosterone had subsided but little, and the remains of the tablets were still palpable under the skin. The advantages of this form of therapy still await further observation and evaluation, although each patient has expressed his willingness to repeated implantation when and if decided upon.

[3] The testosterone tablets were supplied by Schering Corporation, Bloomfield, New Jersey, through the kindness and courtesy of Dr. Max Gilbert.

THE TOLERANCE OF MALE AND FEMALE MICE RESPECTIVELY TO ESTROGENS AND ANDROGENS[1]

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IT HAS BEEN KNOWN for some time that there is present in both the male and female organism the sex hormones of both sexes (1). These have been recently demonstrated to be true androgens (2, 3) and estrogens (4, 5). The functional reason for the presence or the origin of the sex hormone of one sex in the body of the other has not yet been established. There are available, however, certain facts which suggest that in some instances they act as pace-setters and in other instances as checks through their antagonistic action (1, 6). The estrogens in heavy doses are decidedly toxic and indeed bring about pathological changes (6). The androgens on the other hand, have not shown as yet any toxic or pathological properties in the intact animal. In fact they are known to offset many pathological effects of the estrogens (6). On the other hand, in the adrenalectomized rat, the androgens have definite toxic properties with respect to survival (7) and thymus involution (8).

In the course of a study of the relationship of androgens to spontaneous neoplasms, we have made certain observations in line with this antidoting property.

METHOD

The mice used were of the Little dba strain, subline 212, raised from a stock colony obtained from the Jackson Memorial Laboratory. The mice were divided so that there would be as nearly as possible an equal division of litter mates among the various groups.

Injections were begun while the animals were still immature and were administered subcutaneously in the back. The substances used were crystalline testosterone propionate[3] and estradiol dipropionate[4] dissolved in sesame oil so that the daily dose was contained in 0.025 cc. The control animals in every instance were treated with an equivalent amount of sesame oil.

RESULTS

The schema of the experiments are presented in table 1. There were two experiments with testosterone propionate; one in which a 0.20 mg. dose was used (presented graphically, fig. 1) the other in which a 0.40 mg. dose was used showing essentially the same results. Although the weights of the mice were not recorded until 40 days after the beginning of the injections, there are two very important facts to be

[1] This investigation was aided by a grant from the International Cancer Research Foundation administered by Professor John R. Murlin.

[2] Mr. F. E. Korn, Jr., aided in the handling of the mice during a part of the course of treatment.

[3] Perandren (Ciba) and oreton (Schering). The author wishes to express his appreciation to Dr. E. Oppenheimer of Ciba Pharmaceutical Products and Dr. E. Schwenk of Schering Corporation for generous supplies of these substances.

[4] We are indebted to Dr. E. Oppenheimer for this material.

TABLE 1. SCHEMA OF EXPERIMENTS

Fig.	Substance	Daily Dose	At Beginning of Experiment: No.	Av. Wt. Per Mouse	Sex	Age
		mg.[1]		gm.		days
1	Sesame oil	0.025	17	26.0[2]	♂	39 (24–46)
	Sesame oil	0.025	19	23.0[2]	♀	
	Testosterone propionate	0.20	17	26.5[2]	♂	
	Testosterone propionate	0.20	19	20.6[2]	♀	
	Sesame oil	0.025	20	23.5[3]	♂	33 (23–48)
	Sesame oil	0.025	21	20.2[3]	♀	
	Testosterone propionate	0.40	20	22.0[3]	♂	
	Testosterone propionate	0.40	20	19.3[3]	♀	
2	No treatment		21	14.0	♂	41 (33–49)
	No treatment[4]		16	12.5	♀	
	Estradiol dipropionate	0.25	20	13.4	♂	
	Estradiol dipropionate	0.25	18	13.7	♀	
3	Sesame oil	0.025	27	19.6	♂	50 (31–79)
	Sesame oil	0.025	21	15.9	♀	
	Estradiol dipropionate	0.10	24	18.8	♂	
	Estradiol dipropionate	0.10	21	17.1	♀	
	Estradiol dipropionate+ Testosterone propionate	0.10 + 0.40	23	19.6	♂	
	Estradiol dipropionate+ Testosterone propionate	0.10 + 0.40	19	17.4	♀	

[1] The values for sesame oil are in cc.
[2] These values are for 40 days after beginning of experiment.
[3] These values are for 28 days after beginning of injections.
[4] These animals received 0.25 mg. estradiol dipropionate daily 28 days after beginning of experiment

noted. The male treated mice showed a smaller increase in body weight which became more and more evident as injections were continued. Furthermore, there was no alleviation upon decreasing the dose from 6 to 3 times per week.

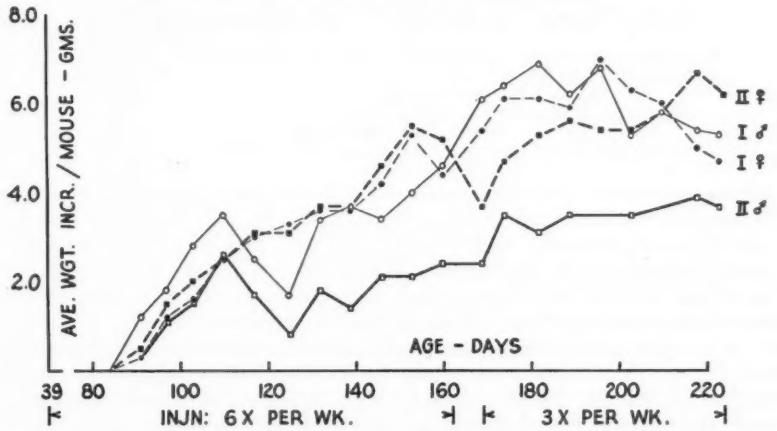

Fig. 1. EFFECT OF TESTOSTERONE PROPIONATE ON GROWTH.
Controls: Males (I♂) o—o, Females (I♀)= ●———●
Testosterone propionate: Males (II♂)= □——□
Females (II♀)= ■——■

This is a positive demonstration of a toxic property in the intact animal attributable to androgens. Previous reports (9) from this laboratory which have been confirmed in man (10) and rabbits (11) have shown that androgens will cause an increase in body weight. The flattening of the growth curve of the experimental males is of the same nature is that obtained normally with age, suggesting that there is a premature ageing of these animals as a result of excessive male hormone. Since the in-

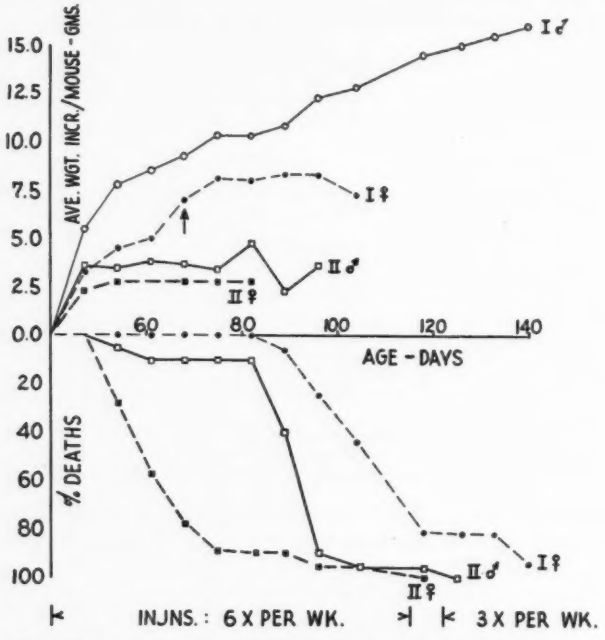

Fig. 2. Effect of estradiol dipropionate on growth and survival.
Controls: Males (I♂) = ○—○
Females (I♀) = ●— — —●
Estradiol dipropionate: Males (II♂) = □—□
Females (II♀) = ■— — —■
Arrow (↑) indicates beginning of injections in female controls

jections were begun while the animals were still premature (24 to 46 days), it may be surmised that the excess of male hormone interfered with the normal development of growth hormones. On the other hand, the retardation might be due to a general toxic effect (7, 8). In contrast with the males, it was found, surprisingly, that the females tolerated the treatment without any apparent effects on body weight. Possibly, the female is able to metabolize better the excess androgen.

A very potent estrogen, estradiol dipropionate, in an excessive dose proved very toxic (fig. 2). This is evident not only in the effect on body weight but also in the death of the animals. Consider first the body weight increase. There was within a week after injection a complete cessation of growth which was more marked in the females. This was confirmed in the case of the latter by beginning injections in their controls 28 days later (arrow in fig. 2). Again there was an immediate cessation of growth in spite of the fact that these animals were older at time of injection. The mortality rate clearly demonstrates the ability of the males to tolerate better the female

hormone. Within 35 days of injection 90% of the females died while it required 56 days to produce the same effect in the males.

The antagonistic action of the two sex hormones suggested a comparative experiment in which an attempt was made to nullify the lethal property of the estradiol dipropionate by testosterone propionate injected simultaneously in the same solution (fig. 3). The dose of the estrogen was reduced to 40% and 4 times as much by

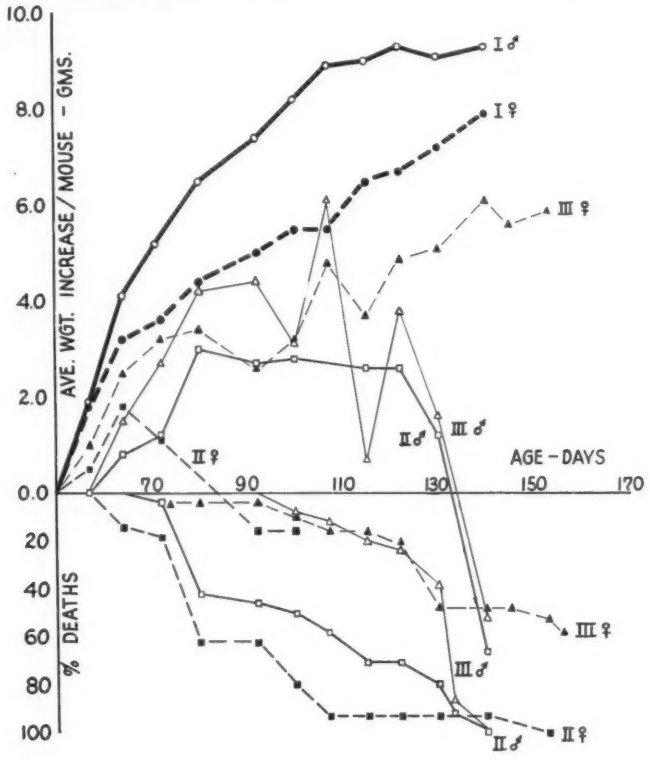

Fig. 3. Effect of estrogen and estrogen plus androgen on growth and survival.
Controls: Males (I♂) = ○—○
Females (I♀) = ●— — ●
Estradiol dipropionate: Males (II♂) = □—□
Females (II♀) = ■— — ■
Estradiol dipropionate plus testosterone propionate: Males (III♂) = △—△
Females (III♀) = ▲— — ▲

weight of the testosterone propionate was added. The weight curves showed the same general effect as in the previous experiment except that there was not a complete cessation of growth until 2 to 3 weeks for the females and about 7 weeks for the males as compared to 1 week after injections with the higher dose of estrogen (fig. 2). The addition of the testosterone propionate to the estradiol dipropionate resulted in a partial but not complete alleviation of the cessation of growth, the weight curves falling between the controls and the estradiol dipropionate treated mice. The mortality (fig. 3) as a result of the estradiol dipropionate injections again was pronounced but required more injections. The same difference in reaction to the

estrogens between males and females was noted, but it was not so great. It is of significance that the testosterone propionate strongly delayed the mortality of the animals. The antidote effect, however, was not sufficient to offset completely the toxic properties of estradiol dipropionate. Furthermore, the males were more susceptible to the residual toxicity than the females.

All of the animals treated with estradiol dipropionate at autopsy showed the typical 'estrinized' effect. The bladders usually were distended and contained considerable chalky precipitate, the kidneys showed definite damage (hydronephrosis) and occasionally stones were found in the kidney pelvis.

Another striking sex difference was noted with respect to hair growth in the mice treated with testosterone propionate. Approximately 1 month after initiation

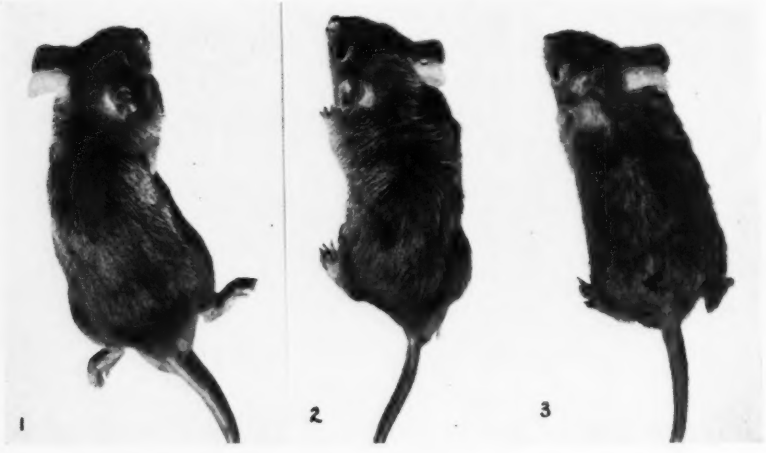

Fig. 4. THESE PHOTOGRAPHS ARE OF REPRESENTATIVE MICE OF EXPERIMENT 1 TAKEN 200 DAYS AFTER INITIATION OF INJECTIONS.

No. 1—Sesame oil treated male.

No. 2—Testosterone propionate treated female.

No. 3—Testosterone propionate treated male.

The control mouse in spite of the sesame oil treatment shows a normal sleek appearance, the androgen treated female shows a slight thinning in the neck-head region while the androgen treated male shows definite hair loss with exposure of the skin. There are noticeable dried blood stains which resulted from fighting. These animals were more aggressive and irritable. The decreased growth of the androgen treated males is also obvious from the photographs.

of injections, the hair of the androgen-treated animals became dull, dry, rough and shaggy, while that of the sesame-oil-treated controls was sleek and normal. Another month later there was definite thinning of the hair to the extent of exposure of the skin. The hair loss in the males occurred along the length of the back and frequently included the head. The ventral portion was affected only slightly and then only in severe cases. Of the animals receiving 0.20 mg. testosterone propionate all the males demonstrated loss of hair with varying degrees of exposure of the skin. Only one of the females showed definite loss of hair and that a small area in the sacro-iliac region. In the mice being treated with 0.4 mg. testosterone propionate there are 5 out of 20 females showing some loss of hair, while all of the males, 20, are affected. However the loss of hair is not so extensive over the surface as in the males receiving the smaller dose of androgen. It seems, therefore, that size of dose as well as the sex of

the animal is a factor. It is noteworthy that the hair loss is localized to the back and the head. This phenomenon cannot be attributed to the administration of the material at this site or the oil, since the controls receiving a similar treatment with only the oil showed no effect and the females receiving a treatment identical to that of the males showed much less effect.

The amount of hair loss has not increased to any notable extent since the above recording. The photographs (fig. 4) show the differences noted above as depicted by an animal from each group of experiment 1 (0.20 mg. testosterone propionate). Since the male and female controls showed no differences only an animal from the male controls (No. 1) is presented. The male (No. 3) and the female (No. 2) represent an average picture of each group.

DISCUSSION

The ability of the female mouse to buffer the toxic properties of testosterone propionate suggested the possibility that the administered androgen was being 'titrated' by an ovarian secretion. Similarly the ability of the males to resist the toxic properties of estradiol dipropionate suggested that the estrogen was 'titrated' by the testicular product. The apparent 'titration' of one sex hormone by the other became more obvious when the two crystalline products were injected and there was a distinct amelioration of the toxic properties of the estrogen by the androgen. Analogous findings with respect to other physiological and pathological properties of estrogens (1, 6) have been noted. It might be surmised, therefore, that the raison d'etre of both sex hormones in each sex is to act as a check and balance system through a process of oxidation and reduction of one hormone by the other. Our present knowledge of the chemistry of the androgens, estrogens and also progestins demonstrates that the forms of all the known sex hormones excreted in the urine seem to be transformation products of less potency than the gonad produced substances (1).

Hitherto there has been no evidence of a toxic property due to the administration of androgens. The toxic properties with respect to body growth and hair growth (consequently possible skin effects) indicate that the androgens, too, when given in physiologically excessive doses will seriously affect the normal economy of the animal.

The loss of hair plus the flattening of the growth curve of the males in a fashion analogous to that obtained with age suggest that there was a premature ageing of these mice. The male parents of these animals at about 10 months of age began to show a slight thinning of the hair in the neck region and also at the base of the tail. This progression is similar to that observed in the androgen-treated animals but was not so marked and occurred at a very much later age. These toxic properties warn against prolonged treatment with heavy doses especially in the case of children.¶

That hair growth is associated with the endocrines has been known (12). The male and female pattern, the hirsutism as a result of adrenal tumor in the female (13) and the feminization in the male (14) are recognized. Abnormalities and changes in hair growth have been noted also in cases of hypothyroidism, gonadal deficiency and pituitary tumors (12). Very recently Schill (15) reported the complete loss of hair in an aged man. Removal of a hypernephroma resulted in restoration of body and scalp hair. Whether the phenomenon of loss of hair in the mice is due to a direct action of the androgen or indirect action through another endocrine organ cannot be stated. The greater effect obtained with the smaller dose emphasizes the importance of dosage in interpreting and expressing results. It is of peculiar significance that the female litter mates of the males were able to better maintain their hair

growth. It is fair to assume, therefore, that a proper amount of estrogen administered simultaneously with the androgen will prevent the loss of hair.

SUMMARY

Each sex of the Little dba mice was better able to tolerate the 'sex hormone' of the opposite sex. Testosterone propionate in daily doses of 0.4 mg. and 0.20 mg. was not fatal but caused a decrease in gain of body weight in the males. Estradiol dipropionate in doses of 0.25 and 0.10 mg. per day was fatal but more so in the females than the males. Testosterone propionate added to the latter in 4 times its quantity was able to nullify in part but not completely the fatally toxic properties of the estrogen.

Testosterone propionate caused a definite and striking loss of hair in the male mice and only a slight effect in females. The controls showed no effect.

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THE COMPOSITION OF BONES OF MICE RECEIVING ESTROGENS AND ANDROGENS[1]

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FOLLOWING the injection of large amounts of estrogens, the marrow cavities of the femurs of mice are invaded and largely replaced by bone (5, 13). Similar changes occurred elsewhere in the skeleton except at the pubic symphyses (4). The changes took place more slowly in males and were prevented by large doses of testosterone (6). This investigation was designed to determine whether these anatomical changes were associated with changes in chemical composition of the osseous tissues.

Determinations of ash, total calcium and total phosphorus were made on the femurs and pelves of control mice and of mice receiving estradiol benzoate, testosterone propionate and estradiol benzoate plus testosterone propionate.

PROCEDURE

The mice of both sexes were of 4 groups: *a*), control animals, some of which received injections of cholesterol; *b*), animals receiving weekly or biweekly injections of 16.6 micrograms of estradiol benzoate; *c*), animals receiving weekly or biweekly injections of 1.25 mg. testosterone propionate and *d*), animals receiving simultaneously both hormones (3). The hormones were all administered subcutaneously in sesame oil.[2]

The innominate bones were broken to separate approximately the ileum from the pubis and ischeum. The pubic and ischeal bones were analyzed and will be referred to further as pelves.

The bones were freed from extraneous organic matter by heating for a few minutes in a dilute alkaline solution. After drying overnight at 100° they were extracted in a Soxhlet extractor with a mixture of alcohol and ether and dried to constant weight. Ash was determined by heating in a muffle furnace for 2 days at 400° C. The ash was dissolved in 5 cc. of 0.1 N hydrochloric acid and made up to a volume of 50 cc. Calcium was determined as the oxalate by a modification of the method of Halverson and Bergeim (7) and phosphorus by the colorimetric method of Fiske and Subbarow (3) with proper control of the acidity. Bogert and Hastings (1) have shown that ashing at a low temperature may result in a slight loss of carbonate but the phosphorus may still be determined by the Fiske and Subbarow method. The calcium oxalate was precipitated in a buffered solution at approximately PH 4.2.

[1] From a thesis submitted by John H. Wentworth to the faculty of the Yale University School of Medicine in candidacy for the degree of Doctor of Medicine, 1939.

Aided by a grant from the Fluid Research Fund, the Anna Fuller Fund of the Yale University School of Medicine, and the Jane Coffin Childs Memorial Fund for Medical Research.

[2] The estradiol benzoate (progynon-B) and testosterone propionate (oreton) were supplied by the Schering Corporation through the courtesy of Drs. E. Schwenk and M. Gilbert.

TABLE I

No. and sex	Age days	Hormone total dose mg.	Weight sample mg. femurs	Weight sample mg. pelves	Weight ash mg. femurs	Weight ash mg. pelves	Weight Ca mg. femurs	Weight Ca mg. pelves	Weight P mg. femurs	Weight P mg. pelves	% ash femurs	% ash pelves	% Ca in ash femurs	% Ca in ash pelves	% P in ash femurs	% P in ash pelves	mg. Ca / mg. P femurs	mg. Ca / mg. P pelves
		Cholesterol																
1	368♂		41.0	24.0	23.0	14.4	8.4	5.0	4.0	2.4	56	60	36.8	35.0	17.6	16.9	2.09	2.08
2	360♂	22.5	46.7	35.8	28.5	20.5	10.6	7.4	5.0	3.4	61	57	37.0	35.9	17.6	16.5	2.11	2.18
3	479♂		41.7	28.8	25.0	15.9	8.4	5.8	3.9	2.8	60	55	33.4	36.5	15.5	17.5	2.13	2.09
4	479♂		46.0	27.0	27.2	15.0	9.6	5.4	4.6	2.6	59	55	35.5	36.3	16.9	17.5	2.11	2.07
5	490♂		32.2	23.5	18.9	13.1	6.4	4.5	3.0	2.2	59	56	33.6	34.4	16.0	16.8	2.10	2.05
									mean, males		59	57					2.11	2.09
6	368♀		49.8	28.7	30.7	18.6	11.4	6.2	5.3	2.8	62	65	37.0	33.0	17.2	15.3	2.15	2.16
7	326♀	20.0	49.8	32.7	31.0	20.1	11.2	7.2	5.2	3.3	62	62	36.0	35.8	16.7	16.6	2.16	2.16
8	317♀		38.8	34.0	24.8	19.4	9.2	6.7	4.2	3.2	64	56	36.9	34.3	16.9	16.8	2.20	2.06
9	370♀	22.5	47.3	29.0	31.1	18.0	10.8	7.0	5.0	3.3	66	62	34.6	38.6	16.2	18.2	2.13	2.12
									mean, females		64	61					2.16	2.12
									mean, both		61	59	35.6	35.5	16.7	16.9	2.13	2.11
									probable error of mean[1]		0.65	0.82	0.40	0.36	0.16	0.18	0.008	0.011
		Estradiol benzoate																
10	121♂	0.10	46.6	22.6	30.9	14.3	11.2	5.2	5.1	2.4	66	63	36.1	36.3	16.6	17.1	2.18	2.12
11	169♂	0.33	51.9	26.1	36.4	17.1	11.4	6.4	5.4	3.1	70	65	31.2*	37.8	14.6	18.3	2.12	2.06
12	249♂	0.45	55.3	29.7	36.7	18.3	14.6	7.6	6.8	3.6	66	62	40.0	41.3	18.4	19.6*	2.17	2.10
13	298♂	0.58	62.7	38.1	43.8	23.7	16.0	8.4	7.2	4.0	70	62	36.7	35.2	16.3	17.0	2.25	2.07
14	348♂	0.67	51.5	25.3	35.5	16.3	11.8	5.9	5.4	2.9	69	64	33.4	36.3	15.1	16.5	2.22	2.06
15	344♂	0.67	73.5	31.2	51.9	19.6	19.4	7.6	8.8	3.6	70	63	37.4	38.5	16.9	18.4	2.22	2.10
16	358♂	0.63	48.7	21.5	33.2	14.7	11.8	5.0	5.5	2.4	68	68	35.4	34.4	16.5	16.7	2.14	2.06
17	350♂	0.73	68.6	27.1	48.6	17.1	17.1	6.2	8.0	3.0	71	63	35.2	36.2	16.5	17.7	2.16	2.05
									mean, males		69	64					2.18	2.08
18	248♀	0.43	58.5	26.2	39.1	15.3	15.2	6.0	7.0	2.7	67	59	39.0	39.2	17.8	17.8	2.19	2.20*
19	270♀	0.47	64.4	29.0	44.5	19.8	16.0	6.4	7.1	2.8	69	68	36.1	32.1	15.9	14.3*	2.27	2.24*
20	394♀	0.42	59.8	28.4	40.8	19.2	15.2	6.7	6.7	3.2	68	68	36.3	35.0	16.4	16.9	2.28	2.06
									mean, females		68	65					2.25	2.06
									mean, both		69	64	36.6	36.6	16.5	17.5	2.20	2.08
									probable error of mean[1]		0.34	0.62	0.45	0.58	0.22	0.17	0.111	0.006

TABLE 1 (*continued*)

No. and sex	Age	Hormone total dose	Weight sample mg.		Weight ash mg.		Weight Ca mg.		Weight P mg.		% ash		% Ca in ash		% P in ash		mg. Ca / mg. P	
	days	mg.	femurs	pelves	femurs	pelves	femurs	pelves	femurs	pelves	femurs	pelves	femurs	pelves	femurs	pelves	femurs	pelves
		Testosterone propionate																
21	408♂	62.50	35.3	24.6	20.3	12.6	7.3	4.4	3.4	2.1	58	51	36.0	35.3	16.5	16.8	2.18	2.10
22	462♂	76.25	34.1	27.6	19.7	14.6	6.8	5.2	3.1	2.5	58	53	34.8	35.3	15.8	17.0	2.20	2.07
23	479♂	78.75	38.5	35.0	24.9	21.4	8.8	7.2	4.3	3.5	65	61	35.2	33.8	17.2	16.5	2.04	2.04
24	479♂	78.75	32.2	19.8	20.4	11.8	6.8	4.1	3.6	2.0	63	60	33.5	34.5	17.4	16.5	1.93	2.09
25	479♂	76.25	37.9	20.8	22.1	12.0	7.6	4.1	3.8	1.9	58	58	34.4	34.4	17.3	15.9	1.99	2.16
											60	57	34.8	34.7	16.8	16.5	2.07	2.09
											1.02	1.32	0.27	0.19	0.19	0.12	0.034	0.045
	Estradiol benzoate	*Testosterone propionate*																
26	252♂ 0.47	35.0	32.9	25.1	21.5	14.5	7.6	5.2	3.8	2.6	65	58	35.1	35.5	17.5	18.3	2.01	1.94
27	233♂ 0.50	36.2	33.1	21.9	19.2	12.1	6.8	4.6	3.4	2.3	58	55	35.7	38.4	17.5	18.9	2.02	2.02
28	337♂ 0.70	105.0	43.8	34.1	24.2	18.2	8.8	6.5	4.1	3.0	55	53	36.4	35.7	17.1	16.6	2.12	2.10
29	327♂ 0.67	100.0	36.1	24.4	19.5	12.1	7.2	4.8	3.5	2.3	54	50*	36.9	39.6	18.0	18.8	2.06	2.11
30	137♂ 0.23	17.5	51.7	35.5	31.4	21.0	11.2	7.4	5.4	3.4	61	58	35.6	35.0	17.0	16.4	2.08	2.13
31	204♂ 0.77	28.8	49.5	31.9	31.5	20.3	11.0	6.7	5.3	3.4	64	64	34.8	33.0	16.8	16.5	2.07	2.00
									mean, males		60	58					2.06	2.05
32	252♀ 0.47	35.0	33.1	22.2	20.4	13.1	7.4	4.8	3.7	2.4	62	60	36.5	36.2	17.9	18.1	2.03	2.00
33	134♀ 0.20	12.0	37.4	28.4	23.0	16.2	8.9	6.2	4.2	3.0	61	57	37.8	38.3	18.4	18.7	2.06	2.05
34	296♀ 0.55	82.5	45.5	33.1	27.9	19.4	10.0	6.8	4.7	3.3	61	59	36.0	35.3	16.9	16.9	2.13	2.08
35	143♀ 0.25	18.8	32.7	18.7	19.5	11.8	6.8	4.1	3.2	2.0	60	63	34.6	34.7	16.6	17.2	2.09	2.02
36	214♀ 0.80	30.0	38.1	23.2	23.8	14.3	8.3	5.4	4.2	2.4	62	62	34.9	37.4	17.4	17.1	2.00	2.18
37	176♀ 0.67	40.0	41.1	26.8	23.4	16.3	8.4	5.8	4.1	2.8	57	61	36.1	35.3	17.5	17.2	2.06	2.05
									mean, females		60	60					2.06	2.06
									mean, both		60	59	35.9	36.2	17.4	17.6	2.06	2.06
									probable error of mean[1]		0.64	0.64	0.16	0.37	0.10	0.18	0.008	0.031

[1] The probable error of the mean, $PE_m = \frac{0.6745SD}{\sqrt{N}}$, where N is the number of cases and $SD = \sqrt{\frac{\Sigma d^2}{N-1}}$, in which Σd^2 is the sum of the squares of the deviations from the mean. Cases marked with an asterisk are more than 2 standard deviations from the mean and are not included in calculating the final SD.

RESULTS

The results are summarized in table 1 and figure 1. The replacement of the marrow cavities by bone in the mice receiving estradiol (fig. 2) is reflected in the higher concentration of ash in the femurs of animals in this group (69%) than in the control group (61%, Significance Ratio 8.2),[3] in the testosterone group (60%, S.R. 8.4) or in the estradiol plus testosterone group (60%, S.R. 12.3). In spite of the poor calcification about the pubic symphyses the concentration of ash from the pelvic bones as a whole was appreciably higher (64%) than in the control group (56%, S.R. 4.9), testosterone group (57%, S.R. 4.8) or estradiol-testosterone group (59%, S.R. 5.6). These changes were significant in spite of the fact that most of the animals in this group were males and the changes in these were less marked than in the females.

TABLE 2

No. and sex	Age days	Estradiol benzoate mg.	Ca/P ratio	
			Endosteal	Periosteal
38♀	339	0.166	2.15	2.09
39♂	504	0.55	2.15	2.13
40♀	279	0.50	2.20	2.15
			Total bone	
41♂	566		2.07	
42♂	579		2.01	

The pronounced difference in the concentration of ash in the males (femurs 59%, pelves 57%) and females (femurs 64%, pelves 61%) of the control group is of particular interest (fig. 1). The bones of the males of this group are similar to those of both sexes receiving testosterone (femurs 60%, pelves 57%) and estradiol plus testosterone (femurs 60%, pelves 59%). The bones of the animals, both male and female, receiving estradiol have a higher ash content (femurs 69%, pelves 64%) than the females of the control group (femurs 64%, pelves 61%) indicating that the estradiol has intensified the female characteristics of the bone.

Since the percentages of ash from the femurs and pelves of the mice receiving estradiol were higher than in any of the other groups it was not surprising to find that the percentages of calcium and phosphorus, on the basis of the weight of the dried extracted bones, was higher in this group. When, however, the concentrations of calcium were calculated as percentage of the weight of ash the concentration of calcium in the femurs of the mice receiving estradiol was significantly higher than in the group receiving testosterone (S.R. 3.4), but not than in the other two groups. A similar relation (S.R. 3.1) existed in the pelves. The concentration of phosphorus in the ash of the femurs of the control animals was less (S.R. 3.7) than in the mice receiving estradiol plus testosterone and the concentration in the ash of the pelves of mice receiving estradiol was higher (S.R. 4.8) than in the group receiving testosterone.

The concentrations of calcium and phosphorus in the ash probably yield less information concerning the changes than the calcium-phosphorus ratios. These are of considerable interest for they show that the ratio in the femurs of the estradiol group is higher than in the control (S.R. 5.0), testosterone (S.R. 3.6) or estradiol testosterone (S.R. 8.2) groups. The ratio in the control group is also significantly higher than in

[3] The significance ratio is equal to the difference between the means divided by the probable error of the means. Differences of means are usually considered statistically significant if the significance ratio is greater than 3.0.

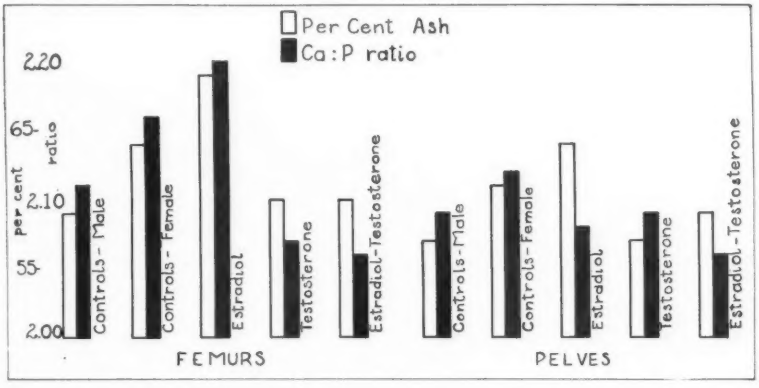

Fig. 1. Percentage of ash and calcium-phosphorus ratios.

the estradiol-testosterone group (S.R. 6.4). In contrast to this the ratios in the pelves are similar in all three groups.

To determine whether the abnormal calcification of the bones of mice receiving estradiol differed chemically from the rest of the bone the following experiments were carried out. The outer or periosteal bone of the diaphyses and epiphyses was chipped away from the central endosteal and less compact bone of 3 mice that had

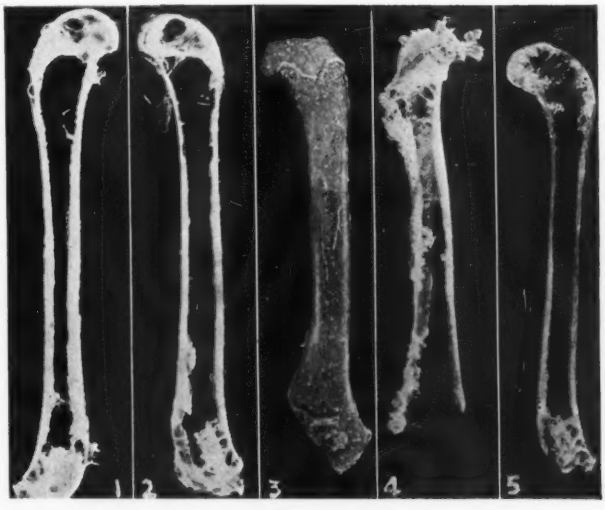

Fig. 2. Ground longitudinal femurs of mice. 1. 6 An untreated female, 368 days of age. 2. 1 An untreated male, 368 days of age. 3. 13 A male that received 0.583 mg. estradiol benzoate in 298 days starting at 51 days of age. The marrow cavity was largely replaced by bone. 4. 22 A male that received 76.25 mg. testosterone propionate in 434 days, starting at 28 days of age. These bones are difficult to grind down. The thin layer of bone in the center is from the opposite diaphyseal wall. 5. 32 A female that received 0.466 mg. estradiol benzoate and 35 mg. testosterone propionate in 207 days starting at 45 days of age. Femurs essentially normal.

received estrogens. This separation was necessarily not quantitative. The two fractions were heated for a few minutes on the steam bath in 5 cc. of 0.1 N hydrochloric acid, made up to volume and filtered to remove any material not dissolving within a short time. The intact femurs from two normal mice were similarly dissolved in acid. Calcium and phosphorus determinations were made and the ratios calculated. These appear in table 2. It is evident that the ratios for two fractions of bone were not appreciably different but were both higher than the ratio in normal bone. If any appreciably higher ratio ever existed in the new bone it may have disappeared since the anatomical changes are comparatively slow. The results indicated also that the lower calcium-phosphorus ratio in normal bone was not primarily due to larger quantities of organic phosphorus compounds in the bone marrow since it was unlikely that the mild acid treatment would have hydrolyzed more than a fraction of these to inorganic phosphorus.

DISCUSSION

The higher ash of female bones is in accordance with the observations of Hammett (8) in normal rats. The above observations suggest that the presence of female hormones or the lack of male hormones in normal animals may be responsible for the difference in the concentration of inorganic constituents in the bones of the male and female skeleton.

The higher ratio of calcium to phosphorus in the ash of the femurs from the estradiol group could obviously be accounted for either by a higher concentration of calcium or by a lower concentration of phosphorus. The lower concentration of organic matter in these bones and consequently smaller amounts of organic phosphorus compounds might tend toward a larger calcium-phosphorus ratio in the ash, but the actual concentration of phosphorus in the estradiol group is not appreciably less than in the control group. The higher calcium-phosphorus ratios in the estradiol group is due principally to the higher concentration of calcium in the ash.

The bones of rats receiving estrogens have been studied by Macola (11). He found that the calcium-phosphorus ratios of the femurs were higher in the treated animals (2.39 compared with 2.10 in the normal animals), however the changes were due to a lower percentage of phosphorus with no change in calcium.

The higher calcium-phosphorus ratios in the femurs of the estradiol group is probably due to deposition of a more basic calcium salt. Kramer and Shear (9, 10) have shown that both in primary and in certain pathological calcifications the calcium-phosphorus ratios are significantly above the ratios in normal bone. If the formula for the inorganic part of bone is approximately $CaCO_3$: n $Ca_3(PO_4)_2$ then in the bone of mice receiving estradiol the value of n is smaller than in normal bone, that is the proportion of calcium carbonate is larger.

It is interesting to note that chemically the bones of the group receiving testosterone were essentially normal and that in the group receiving both estradiol and testosterone the testosterone apparently prevented the chemical changes from taking place.

The mechanism whereby estrogens effect the changes in the skeleton of the mouse are unknown as well as the means whereby they are prevented by androgens.

Parathormone, vitamin D and related sterols are known to alter calcium metabolism and in certain instances the structure of bone. Burrows (2) observed an increased deposition of osseous tissue in the bones of rats receiving parathormone over extended periods. In birds profound changes in the skeleton and serum calcium occur during the reproductive cycles in the female and these changes can be duplicated by the injection of estrogens (12).

SUMMARY

The continued administration of large doses of estradiol benzoate to mice produces significantly higher concentrations of inorganic substances in the femurs and pelves than in control mice or mice receiving testosterone propionate or estradiol benzoate plus testosterone propionate.

The ratio of calcium to phosphorus in the femurs, but not in the pelves, of the mice receiving estradiol benzoate is significantly higher than in the other groups.

The higher ash content of the untreated females as compared with the untreated males indicates the influence of the animals' own hormones on skeletal growth.

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THE MECHANISM OF ESTRIN THERAPY IN THE RELIEF OF DYSMENORRHEA[1]

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THE EXACT CAUSE of pain in most cases of so-called essential dysmenorrhea is still far from clear. Estrin therapy has been widely used and satisfactory results in as high as 70% of the cases (1), have been recorded. The purpose of this paper is to report that within certain well-defined limitations of procedure the relief from such therapy is not only dramatic and sure but associated with a consistent change in ovarian function.

The nature of this change was quite unexpected in view of certain facts from the literature pointing in another direction. Thus, Novak and Reynolds (2) ascribed the severe pelvic cramps of dysmenorrhea to a relative deficiency of progesterone, the hormone of the corpus luteum. In favor of such a thesis is the apparent function of the corpus luteum during pregnancy in certain animals to protect the products of conception by rendering the uterus unresponsive to stimuli (3). Knaus (4), furthermore, reported that during the latter half of the human menstrual cycle progesterone produces an atonic uterus, refractory to stimulation with pituitrin, and Elden and Wilson (5) advocated progesterone for mitigating the cramps of dysmenorrhea.

In the observations to be recorded, on the contrary, the presence of an apparently normal corpus luteum seemed to be a prerequisite for the occurrence of cramps. Even more important, estrin therapy was apparently followed by a cramp-free period only when it prevented the development of a functioning corpus luteum during that cycle. In fact, dysmenorrhea could be induced in a patient having anovulatory periods by administration of a sufficient amount of progesterone. The data to be reported are entirely consistent with the statement of Wilson and Kurzrok (1) that "the presence of functional dysmenorrhea indicates the occurrence of ovulation" and offer corroborative evidence to their conclusion that "only patients with corpus luteum function can have (functional) dysmenorrhea."

No explanation as yet suggests itself as to why, in certain individuals, the occurrence of a normal physiological process such as ovulation should be the basis for cramps pathological in their severity. Almost all normal women are conscious during menses of uterine motility at least, and moderate to severe cramps are not called dysmenorrhea until the patient consults a doctor. Hence, the dividing line clinically between the normal and the pathological is drawn only by the patient's own individual sensitivity to pain. But since anovulatory bleeding induced in these patients with estrin is painless, the implication of ovulation as the necessary basis for dysmenorrhea seems apparent.

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[1] These studies were aided in part by a grant from the National Research Council.

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METHOD

This study is based on a series of 25 cases. These patients with one or two exceptions had severe, disabling dysmenorrhea, frequently forcing them to miss one to three days from their work. Care was taken in obtaining case histories and in making physical examinations. Signs of delayed maturing such as general or sexual underdevelopment were noted. However, no attempt was made to select cases of any one group or type; the treatment was tried on consecutive cases, only the few that presented probably organic pathology as a factor in the pain being eliminated. No other treatment, glandular, medical, dietary or psychiatric, was employed during the time these patients were given estrin. Although it was fully realized that numerous factors might be involved in the etiology of dysmenorrhea, the only purpose in this study was to find out if there was a hormonal common denominator.

Estrin therapy for dysmenorrhea was first inaugurated in this Clinic in order to test out the theory advanced many years ago by Schultz (6) that hypoplasia of the uterus is the chief cause of the cramps. Dysmenorrhea is frequently associated with uteri often described as 'infantile,' 'hypo-developed' or 'immature.' Since estrin causes a marked increase in the growth and vascularity of the pelvic organs of immature animals (7) it seemed reasonable to suspect that the relief reported by others from this hormone could be ascribed to a similar growth response in human beings. It was decided, accordingly, to give massive doses of estrin between menses on alternate months over a long period of time. It was felt that untoward results from large doses were not to be feared as long as the treatment was not continuous.

Throughout this study an oily solution of estradiol benzoate (progynon-B),[2] one cc. containing 1.7 mg. (10,000 R.U.), has been used. This dose was injected intramuscularly every third day. A series of injections consisted of from three to fourteen.

RESULTS

It was soon apparent that the immediate result of a series of such injections depended entirely on how soon in the month the injections were started. If the first of a series of 6 to 10 or 12 injections was given within the first week after onset of menses, the next period was invariably free from uterine cramps. If the series was not started until 2 weeks after onset of the previous flow, there was no change in the pains during the subsequent bleeding. It was next noted that although a completely cramp-free period followed a course of treatment if started early enough in the cycle, yet the next menses, when no estrin had been given, was as painful as ever. The effect was characteristically an 'all-or-none' one. The dependence of this result on the timing of the injections during the cycle suggested that the effect might be related to the action of estrin upon ovulation.

It was decided then to take an endometrial biopsy shortly before a catamenia on all new cases; to give a series of injections immediately following the period; to take a second biopsy before the oncoming period; and finally, after allowing the next month to go by without treatment, to obtain a third premenstrual biopsy. Many of our patients were young girls in whom biopsies were out of the question, but a sufficient number have now been made to furnish strong evidence that the immediate relief from estrin treatment depends on suppression of ovulation.

It has been possible to procure 15 premenstrual 'control' biopsies from these 25 patients with essential dysmenorrhea. All of these specimens, taken before treatment and directly before a painful period, showed a normal secretory endometrial pattern. We have considered this to be evidence that ovulation had taken place. There have been 9 biopsies taken after a course of injections within a few days of the subsequent flow. All 9 of these showed a proliferative endometrial pattern, and the bleeding

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[2] We are indebted to Dr. Gregory Stragnell and Dr. Erwin Schwenk of the Schering Corp., N. J., who supplied the necessary quantity of progynon-B and proluton (v. infra) for this study.

following the treatment was in each case free from cramps. Thus, every biopsy that was taken before a painful period showed evidence of ovulation, whereas every biopsy obtained after treatment and before a cramp-free period showed no evidence of ovulation. When it was possible to take biopsies in 3 consecutive months it was evident that estrin treatment one month did not inhibit or repress the occurrence of ovulation the next month.

A clinical study of these cases over a period of 6 to 18 months shows that 82 courses of injections have been given. In 63 of the periods directly following such treatment, the patients were free from cramps. An analysis of the 19 times the injections were not followed by relief provides the following 4 additional points.

(*a*) *Timing of injections.* Although a course of injections started on the 8th or even the 10th day after onset of the previous period may give relief, the 6th day seemed to be the

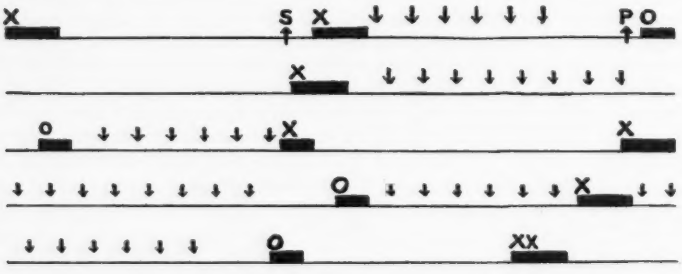

Fig. 1. Each line represents 2 months. X = cramps; O = no cramps; S = secretory endometrium found by biopsy; P = proliferative endometrium; oblong blocks indicate days of catamenia; arrow indicates day on which 1.7 mg. of estradiol benzoate was administered. It should be noted that on the 2nd and 4th lines there were intervals of 14 days and 13 days respectively between two consecutive courses, which allowed maturation of a follicle to occur and resulted in cramps. However, a 3rd consecutive course started at the end of the 4th line resulted in suppression of ovulation just as a first course would and for the same reason. At onset of cramps with the last period recorded on the 5th line, the patient was given 2 mg. of progesterone with no relief whatsoever (XX).

latest which would give dependable results. Two of the failures could be ascribed to starting injections too late.

(*b*) *Number of injections.* When a course of 6 injections every 3rd day was started on the 6th day after beginning of the period, the next flow occurred at about a 4-week interval. If more were given (up to 12 or 14 were tried), the flow was delayed until about a week after the last treatment, and was equally cramp-free. In attempting to ascertain the minimum number of injections that would give relief, it was found that less than 5 was rarely enough, that 5 injections were sufficient more than half the time, but that 6 was the optimum number. Eight of the failures were during months when 5 or less treatments were given.

(*c*) *Consecutive treatment.* In general, as described above, estrin was given on alternate months. This decision in the first place was merely arbitrary. In a few patients we tried repeating the same treatment with the same timing for 2 consecutive months. As a rule we found that cramps returned on the 2nd month in spite of the repetition of the injections. Five of the failures came in the 2nd consecutive month of treatment. This will be discussed more fully below.

(*d*) *Induction of cramps with progestin.* In a few patients who had repeatedly responded to treatment in the past, the optimum number of estrin injections was followed by daily injections of 5 mg. of progesterone (proluton) for 5 days. Although they had been entirely free from cramps previously after the estrin alone, the progesterone caused a return of cramps in 2 out of 3 cases.

Of the 19 failures, therefore, adequate explanation was found 17 times. If these 17 are subtracted from the total of 82 courses of injections there are left 65 courses of optimum treatment followed in 63 cases by a cramp-free period. Twice only in this series has there been unexplained failure to obtain complete relief from pain after the most favorable type of treatment.

In figure 1 an actual record from a representative patient (M.G.H. #4089) is graphically reproduced to illustrate some of the points discussed above.

DISCUSSION

The evidence from endometrial biopsies has led to the conclusion that ovulation is a sine qua non of all types of essential dysmenorrhea. It seems probable that estrin prevents maturation of a graafian follicle through its known antagonistic action on the follicle stimulating hormone (FSH) of the anterior pituitary (8). The continued administration of estrin, however, causes the endometrium to proliferate, and on cessation of treatment, bleeding takes place from this proliferative tissue. Perhaps this flow, which occurs usually 5 to 8 days after the last estrin injection, should be called anovulatory bleeding or 'estrin-withdrawal' bleeding rather than menstruation. It seems clear that the inhibition to the pituitary must take place before the ovarian follicle has received an adequate growth stimulus by FSH. When injections were given 10 or 12 days after the onset of menses, it was found impossible to prevent ovulation. The same mechanism appears to explain why by the above method it was impossible to suppress ovulation 2 months in succession. On withdrawal of estrin, FSH is released to stimulate follicular growth. Since anovulatory bleeding appears approximately one week or more after the last injection, it is evidence that by the sixth day after onset of this bleeding, about two weeks has elapsed for possible growth of the new follicle. When further estrin is given at this time it is not possible to prevent ovulation in that cycle, for the follicle has already developed too far towards maturity. It is quite plain, therefore, that in the suppression of ovulation in any given cycle, the timing of estrin injections is of primary importance.

We do not know as yet whether ovulation is necessary for cramps because the endometrium is made secretory in type, or because the luteal hormone itself has a direct action on the myometrium. Browne and Venning (9) have shown that normally all demonstrable progesterone is apparently eliminated about two days before the menses. This suggests that the cramps must be due to some result of progesterone action such as a secretory endometrium, rather than to its presence itself in the system. In our experience, progesterone given for dysmenorrhea at the onset of menses has not helped and occasionally has made cramps worse.

The present study has made us skeptical of the beneficial results to be derived from progesterone treatment of habitual abortion or threatened miscarriage. On the other hand it is quite possible that the luteal hormone has a different myometrial effect in pregnancy from that during the menses. Moreover, recent investigations (10) have suggested that a uterine contraction is not always simultaneous with the subjective sensation of a cramp. It is therefore possible that the dysmenorrhea associated with ovulation may be on some other basis than that of severity of uterine contractions.

Clinical considerations. The principles found in this study have application in problems other than dysmenorrhea. Thus, the presence of regular menstrual cramps in a case of sterility is good evidence that ovulation is taking place. In metropathia hemorrhagica, the ovaries characteristically show no evidence of normal ovulation, cramps are absent, and bleeding is always from a proliferative endometrium; the

recurrence of cramps is, it is believed, good evidence of the reestablishment of ovulation and therefore of a remission from the disease. Some young girls after 1 or 2 years of crampless periods suddenly develop dysmenorrhea; it is probable that the first periods were anovulatory and that the cramps indicate the start of normal ovarian function with formation of a corpus luteum. Conversely, if one had reason to attribute any symptoms to the presence of a corpus luteum (e.g. acne) or to ovulation (e.g. ovulation cramps) one could try a therapeutic test with estrin therapy and see whether the symptoms disappeared.

End results of treatment. In a few of the patients reported in this study who have received estrin on alternate months for at least a year, there has appeared to be a gradual improvement in the severity of the cramps in the months when no injections were given. It is as yet too early to come to any conclusions concerning the permanent value of this therapy. It seems probable that in some of these cases there may be produced sufficient uterine growth to result in a permanent mitigation of the pain.

SUMMARY

In a patient with essential dysmenorrhea, a course of estrin therapy, if administered in accordance with certain specifications, will result in the absence of dysmenorrhea during the following but not during subsequent menses.

To produce this result, the course of estrin therapy should be started about the sixth day of the cycle and should be of the order of magnitude of 1.5 mg. of estradiol benzoate intramuscularly every 3 days for 6 injections.

Such therapy prevents the formation of a secretory endometrium as demonstrated by endometrial biopsy presumably due to an inhibitory action of estrin on gonadotropic hormones and hence on ovulation.

From this and other collateral clinical evidence it is concluded that essential dysmenorrhea cannot occur in the absence of a secretory endometrium.

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THE RELATION OF HYPEREMIA TO THE ACTION OF ESTRIN

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ALTHOUGH THE course of events following the administration of estrogenic substance is well known, the mechanism of its action is but partially understood. It has long been recognized that the complete estrous phenomenon always includes hyperemia and, indeed, hyperemia is usually the first noticeable effect of estrin administration on the uterus of the castrate animal. A number of investigators (1, 2, 3) have raised the question as to whether the hyperemia is primary or secondary to the other phenomena associated with estrus. In other words, does estrin directly stimulate the cells of the uterus and vagina, the hyperemia being secondary to the increased metabolic activity of the tissues? Or does estrin act by directly increasing the blood supply to the uterus and vagina, the stimulation of the cells being secondary to the hyperemia? We have attempted to answer these questions by observing: (*a*) the effects of estrin on the uterus and vagina of castrated animals under conditions in which hyperemia was partially or completely prevented; (*b*) the effects of a nonspecific hyperemic agent on the uterus and vagina of castrated animals.

Uterine Effects of Estrin When Hyperemia Is Prevented

Our procedure was based upon the recent demonstrations that the hyperemic effects of estrin are accompanied by the liberation of acetylcholine in the uterus (4) and that the initial hyperemia following estrin administration can be temporarily inhibited by a dose of atropine (5). By trial and error in preliminary experiments, we attempted to determine the size and frequency of atropine dosage necessary to maintain a continuous parasympathetic inhibition for the period (48 hours) coinciding with the development of maximal estrin action. Our problem was complicated by the fact that the mice which we used appeared to be markedly resistant to atropine. Whether the doses and times we finally selected (5 to 8 mg. atropine sulfate per mouse, given 3 times in 48 hours) were really sufficient to cause complete and continuous atropinization, it is difficult to say. However, this appeared to be the largest feasible dosage since it killed many of our animals. The data which we are presenting are based only on those animals which survived from the experiment to the times at which they were killed for examination.

Sixty-four adult female mice weighing 18 to 24 gm., were castrated. Thirteen days later, all were given a subcutaneous injection of 10 R.U. dihydroxyestrin benzoate in oil.[2] The animals were then divided into 2 groups. Counting the estrin injec-

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] We are indebted to Dr. Gregory Stragnell of the Schering Corporation for the supply of this material.

tions as zero time, one group received subcutaneous injections of atropine at 0, 3 and 25 hours. Equal numbers of animals from this and from the group which had received only estrin were killed for examination at 9, 24 and 48 hours respectively. The uteri of these animals were examined for changes in hyperemia, distention, water accumulation and proliferation. Hyperemia was determined by gross and histological examination; the proliferative changes were studied histologically and by dry weight increase. The distention and water accumulation were measured objectively in the following manner. Each uterus was removed intact and its total weight, including the fluid content of the uterine cavity, was determined immediately. The organ was then cut open, its surfaces quickly dried by pressing between filter papers, and reweighed. The difference between these 2 weights was used as a measure of uterine distention. The uterus was then desiccated to constant dry weight. The difference between the wet and dry weights was used as a measure of water accumulation in the uterine

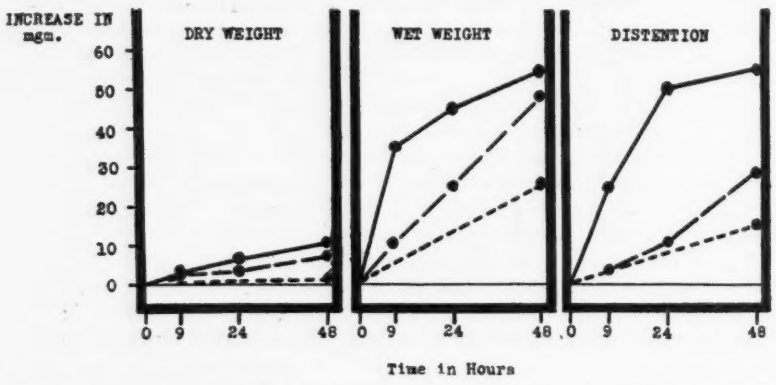

Fig. 1. THE AVERAGE INCREASE IN MILLIGRAMS IN THE DRY WEIGHT, WET WEIGHT AND DISTENTION OF THE CASTRATE MOUSE UTERUS, CAUSED BY ESTRIN (●————●), ESTRIN PLUS ATROPINE (●— — —●), AND YOHIMBINE (●------●) RESPECTIVELY.

tissues. Although our determinations of distention by this method are subject to marked variations, they do avoid the necessity of evaluation by subjective description.

The uteri from the mice which received estrin alone showed definite gross hyperemia within 24 hours. In 48 hours the hyperemia was very marked. The uteri from mice which had received atropine as well as estrin showed practically no gross hyperemia within 24 hours, and only slight hyperemia in a few cases at the end of 48 hours. Histologic examination confirmed this difference. There was a definitely decreased vascularity of the muscular and endometrial layers in the uteri of the atropine treated animals.

Figure 1 illustrates the quantitative differences in distention, wet weight and dry weight increases due to the effects of atropine upon estrin action. It will be noted that atropine, while markedly inhibiting uterine distention, only slightly inhibited the increase in wet weight caused by estrin. This inhibition of the wet weight increase by atropine is probably due to the reduction in the amount of intravascular fluid. It will be further seen that atropine inhibits the increase in dry weight caused by estrin. Since this data gives only composite values, histologic examination was utilized to determine the effects of estrin on the hypertrophy and proliferation of the various uterine elements responsible for the increase in dry weight. These findings

are illustrated by figures 2–5, and summarized in table 1. It may be seen that atropine did not significantly influence the action of estrin on the endometrial lining epithelium. However the atropine effect on the uterine glands and on the size of the stromal cells was somewhat more marked, although it bore no direct relationship to the extent to which hyperemia was inhibited. The inhibiting effect of the atropine on the estrin stimulation of the muscularis, was in proportion to the inhibition of hyperemia.

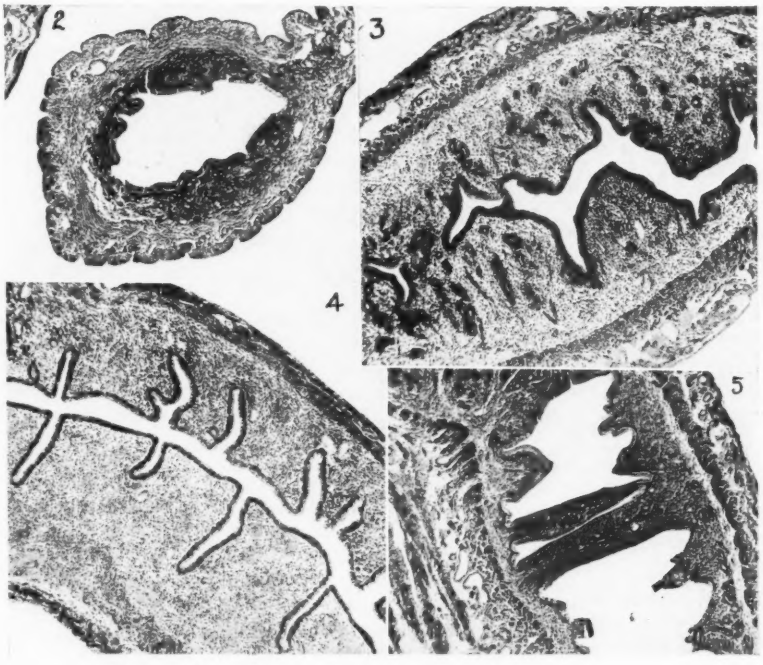

Fig. 2. CASTRATE UTERUS. Hematoxylin-eosin preparation. ×50. Fig. 3. ESTRIN-TREATED UTERUS Hematoxylin-eosin preparation ×42. Note the dilatation of the vessels, the increase in intercellular material, the hypertrophy of the epithelial lining cells and of the endometrial glands, the increase in size of the stromal cells and of the fibers of the muscularis. Fig. 4. ESTRIN+ATROPINE-TREATED UTERUS. Hematoxylin-eosin preparation ×42. Note the lack of dilatation of the vascular channels, yet the moderate amount of intercellular material, the distinct hypertrophy of the epithelial lining cells as compared to castrate, and the moderate hypertrophy of the glands. Fig. 5. YOHIMBINE-TREATED UTERUS. Hematoxylin-eosin preparation ×42. Note the dilatation of the vascular channels, yet the lack of marked change in the lining epithelium. Note also the unique dilatation of the glands lined by small cuboidal cells.

It is evident from these results that the effect of estrin on the uterine lining epithelium is not secondary to hyperemia, since estrin exerts its usual action in the absence of increased vascularity. It appears, however, that maximal hypertrophy of the endometrial stroma, of the endometrial glands, and of the muscularis is dependent upon hyperemia. On the other hand, while hyperemia appears to be largely responsible for distention, it has only a minor influence over water accumulation. This may mean that those effects which were inhibited by atropine, are secondary to hyperemia, or that the specific action of estrin on these uterine reactions is enhanced by increased vascularity. These conclusions were confirmed and extended by our results with a non-specific hyperemic agent in the absence of estrin, as follows.

TABLE 1

	Estrin	Estrin+Atropine	Yohimbine
Lining epithelium of endometrium	Marked increase in depth and moderate increase in No. of convolutions as compared to castrate. Cells are high columnar with increased amount of granular cytoplasm as compared to the small cuboidal cells of castrate.	Varying, moderate to marked increase in No. and depth of convolutions as compared to castrate. Cells are high columnar with increased amount of granular cytoplasm.	Only slight increase in depth and No. of convolutions as compared to castrate. Cells are high cuboidal to low columnar, being only slightly larger than in castrate.
Endometrial glands	Glands much larger than in castrate. Cells are high cuboidal to low columnar with much granular cytoplasm. Some of the lumina are obliterated by the enlarged cells.	Glands larger than in castrate; in some cases less than those in estrin. Cells varied from medium cuboidal to low columnar with corresponding variation in the amount and granularity of cytoplasm. Variation could not be correlated with the extent of hyperemia.	Glands are markedly dilated (not seen in any of the other treated uteri). The cells are cuboidal resembling those in the castrate.
Endometrial stroma	Stromal cells are larger than in castrate, and much more loosely packed, being separated by a serum-like material. The blood vessels are markedly dilated as compared to castrate.	Various changes seen. In some cases stromal cells, slightly larger than in castrate with slight amount of intercellular material. In others, however, approached picture seen in estrin.	Stromal cells are larger than in castrate but distinctly smaller than in estrin. Intercellular material likewise between castrate and estrin.
Muscle	Increase in size of the muscle fibers as compared to castrate.	Size of the muscle fibers varied with the extent of hyperemia. The greater the inhibition of the hyperemia, the greater the inhibition of the hypertrophy. In some cases muscle fiber approaches that seen in castrate.	Moderate increase in size of the muscle fibers as compared to castrate. Less than in estrin.

Uterine Effects of Hyperemia in the Absence of Estrin

In order to study the uterine effects of hyperemia in the absence of estrin, we attempted to simulate normal parasympathetic activity by the use of prostigmine. However, this procedure was complicated by the fact that our mice proved to be extremely sensitive to this drug, and were killed by doses as low as 6γ. The administration of prostigmine in sub-lethal doses (1 to 6γ) by various routes, did not significantly affect the castrate uterus. After preliminary experimentation, yohimbine was selected as our non-specific hyperemic agent.

Sixteen adult female mice, weighing from 18 to 24 gm., were castrated. Eight to thirty days later each mouse was given yohimbine subcutaneously. Some received a single dose of 1 mg., others received 2 ½ mg. doses at various intervals.[3] All the animals were killed for examination 48 hours after the single or the first yohimbine injection.

The effects of yohimbine were consistent in all of the above animals, regardless of the manner in which it was administered. On macroscopic examination the uterine blood vessels were engorged and the uteri were markedly distended, over the cas-

[3] The administration of more than 1 mg. yohimbine to our animals produced toxic manifestations and killed some. None of these animals are included in our results.

trate level. The pink color in the uterine wall in the yohimbine-treated animals, however, indicated a lesser degree of hyperemia as compared to the deep red color of the uterus of estrin-treated animals. This difference was confirmed by histologic examination which revealed a lesser vascularity in the muscular layer in the yohimbine-treated animals as compared to the estrin-treated mice; however, the uteri of the former group were far more vascular than castrate or 'estrin plus atropine' uteri.

Figure 1 illustrates the quantitative effect of yohimbine on distention, and on wet weight and dry weight increase. It will be noted that although yohimbine affects a significant increase in distention over the castrate level, it does not approach the estrin level of distention. Yohimbine caused a definite increase in uterine wet weight, although this was only one-half of that found after estrin injection. While the amount of intravascular fluid almost approached that found in estrin uteri, there was no corresponding increase in the amount of intercellular fluid as evidenced by histologic examination. Examination of the dry weight increase produced by yohimbine reveals only an insignificant rise above the castrate. Histologic examination (fig. 2–5; table 1) showed that yohimbine had only a slight hypertrophic and proliferative effect on the lining epithelium and the uterine glands. However, it may be noted that yohimbine produced a unique effect on the glands in that their luminae were tremendously dilated although their lining cells closely approached those seen in castrate uteri. The endometrial stromal cells and the muscle fibers were moderately increased in size by yohimbine.

These results obtained with hyperemia alone confirm those obtained with estrin and atropine, in showing that the hyperemic effects of estrin may account for endometrial stroma cell and muscle fiber hypertrophy. Since yohimbine did not reproduce the effects of estrin on the uterine glands, it must be concluded that this effect resembles the proliferation of the uterine lining epithelium in being a more specific and direct action of estrin. It appears that distention can be at least partly accounted for by hyperemia, since hyperemia in the absence of estrin did produce this effect. Correlation of our wet and dry weight determinations with histologic detail showed that hyperemia can only account for a small increase in intra- and intercellular water. Hence, it appears that the increased tissue water found following estrin administration is produced by a more specific effect of estrin.

These interpretations may be criticized on the grounds that yohimbine produced an insufficient hyperemic effect and that atropine only partially inhibited the hyperemic action of estrin. From this point of view, one might suppose that, had yohimbine produced maximal hyperemia, a complete uterine estrous effect might have been obtained; and had atropine produced complete inhibition of hyperemia, the complete estrous effect might have been abolished. In other words, uterine estrus might depend solely on the production of hyperemia. But, if this were so, one should expect that the inhibition or stimulation of atropine and yohimbine respectively, would affect the various estrous phenomena in a given animal to approximately the same degree. This is not the case as is evidenced by the following results. (*a*), Yohimbine produced moderate myometrial hypertrophy, partial endometrial stromal changes with minimal epithelial changes, and (*b*), atropine while partially inhibiting myometrial and stromal changes produced only negligible inhibition on the lining epithelium. This lack of correspondence indicates that our results cannot be explained on the basis of hyperemia alone.

This distinction between the specific and the hyperemic effects of estrin is further emphasized by the fact that yohimbine had no effect on the uteri of mice castrated more than 40 days previously, nor upon the involuted uteri of mice with atrophic ovaries. Since estrin exerted its usual actions under both these conditions, it is ap-

parent that the specific effects of estrin are more fundamental than its hyperemic effects, for the former do not require the presence of the mature and non-involuted organ.

Relation of Hyperemia to Vaginal Cornification

The effects of the above procedures on the vaginal mucosa of our castrate mice were observed by the smear technic of Allen and Doisy (6). The results are summarized in the following table.

TABLE 2

Procedure	No. of animals	No. of animals showing typical vaginal cornification 48 hours after treatment
Administration of:		
Estrin	8	8
Estrin+Atropine	8	0[1]
Yohimbine	16	13

[1] At this time the 8 animals, the smears of which had shown no cornification at the end of 24 hours, did show many cornified cells. But there were also many leucocytes and much debris present.

It is clear that atropine prevents the usual action of estrin upon the vaginal mucosa, and that yohimbine causes cornification typical of estrin in the vaginal smear of castrate mice. These results indicate that the effect of estrin on the vaginal mucosa is not specific, but is secondary to its vascular effects. This may serve to explain the well-known fact that vaginal cornification may follow mechanical irritation (7) of the vaginal mucosa.

SUMMARY AND DISCUSSION

Our results show that the effects of estrin may be divided into two groups (*a*) those specific actions of estrin which do not depend on hyperemia, and (*b*) those actions which are secondary to the production of hyperemia, and which are associated with the acetylcholine-liberating action of estrin. Distention seems to be greatly dependent upon hyperemia, since this effect was inhibited by atropine and duplicated by yohimbine. However, it is possible that distention depends in part on the specific effect of estrin on the uterine cells, since some distention occurs when the hyperemic effect of estrin is inhibited by atropine. The idea that distention depends solely on hyperemia is also not in accord with the finding that distention is not always associated with hyperemia, or hyperemia with distention in normal animals (8). The accumulation of tissue water appears to be due mainly to the specific action of estrin. Although, hyperemia plays a rôle in this water accumulation, it appears to be slight, as compared to the direct action of estrin on the uterus.

The proliferation and hypertrophy of the cells of the lining epithelium and the hypertrophy of the endometrial glands depend chiefly on the specific action of estrin, although it appears that some hyperemia is necessary for maximal growth. The only uterine elements which can be stimulated by hyperemia without the specific action of estrin are the endometrial stromal cells and the muscle fibers. This of course does not imply that there is no direct effect of estrin upon these elements.

The fact that vaginal cornification is not a specific effect of estrin is important because of the wide application which has been made of the vaginal smear technic in the biological assay of estrogenic substances. It must be evident that the vaginal smear alone cannot be regarded as a reliable index of true or complete estrogenic action, when obtained with an unknown substance. Nor can the spontaneous occur-

rence of vaginal cornification be accepted as an index of normal ovarian function. It has been possible to observe apparently normal cyclic vaginal cornification in animals which were found upon laparotomy to exhibit atrophic ovaries and uteri (9). These observations must be kept in mind when one attempts to evaluate the recent clinical applications of the vaginal smear technic to the study of human ovarian function (10, 11).

But what is perhaps the most important clinical application of our present results is the fact that such a large proportion of the estrous phenomenon in animals is dependent upon hyperemia. It may be assumed that the rôle of hyperemia is probably even more important in the phenomenon of periodic menstrual bleeding in the human female. Since this hyperemia is presumably also under the influence of at least the parasympathetic nervous system, it is not difficult to understand the well-known profound influence of mental, emotional and physical strain upon the menstrual history of women. It is possible that certain cases of primary amenorrhea and other functional disturbances, which are now generally ascribed to endocrine deficiencies, may in fact exist despite normal hormone secretion, and be due to abnormally decreased or increased vascular responsiveness. If this be so, our therapeutic approach in such cases might better be along pharmacologic rather than along endocrine lines.

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EXPERIMENTAL STUDIES ON THE MENSTRUAL CYCLE OF THE BABOON (PAPIO PORCARIUS). VI. THE EFFECT OF PROGESTERONE UPON THE FIRST PART OF THE CYCLE IN NORMAL FEMALE BABOONS[1]

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IN A PREVIOUS study (1) it was shown that progesterone when repeatedly given in the first part of the cycle of a normal adult female baboon the turgescent perineum underwent deturgescence very rapidly and returned to its resting state. This resting condition could be maintained over a period the length of which was determined only by the administration of the progesterone. Further it was shown that if the total quantity of the hormone exceeded 15 mg. bleeding invariably occurred after withdrawal, the exact time of the onset of bleeding being determined purely by the quantity of progesterone used.

If the total amount of progesterone happened to be less than 15 mg. then, after its induced resting state, the turgescent perineum rose rapidly without any microscopic evidence of bleeding. This experimentally affected cycle was invariably a prolonged one. The conclusion was arrived at that in a normal adult baboon deturgescence is a positive phenomenon initiated by progesterone or an allied substance.

The present paper is a further study of the effect of progesterone on the turgescent perineum of the normal baboon. Its object was first, to determine the minimal amount of progesterone given in a single dose, necessary to overwhelm the turgescence produced by the baboon's own ovarian hormones and to cause deturgescence; second, to discover whether it was not possible by more massive dosage to cause premature bleeding with a single injection of progesterone, and if so, to determine the minimal quantity of progesterone necessary to produce that reaction; third, to ascertain in those cases where progesterone was insufficient to cause bleeding, but sufficient to initiate deturgescence, what effect these quantities of the corpus luteum hormone might have on the subsequent course of the experimental cycle.

MATERIAL

All the baboons were normal uncastrated animals, whose cycles had been observed for at least 6 months and many over a number of years. These baboons all had their complete permanent dentition and were therefore adult.

The progesterone used in this study is manufactured and sold by the Schering Corp. as proluton.

METHODS AND RESULTS

The changes in the size of the perineum have been followed by daily measurement in the manner described previously (2, 3). The maximum width of the perineum there shown to be most sensitive and reliable has continued to be used as an indicator.

[1] This investigation was aided by grants received from the National Research Council and Board (South Africa) and from the Royal Society of London.

The minimal amount of progesterone necessary to produce perineal deturgescence in the first or turgescent part of the cycle i.e. the follicular phase was determined in experiments 1–4.

Experiment 1 (Table 1). *Baboon* 1 with a 38-day-cycle was given 0.5 mg. progesterone on the 11th day of the cycle. There was no measurable change in the perineum and bleeding occurred normally on the expected date. Thus 0.5 mg. progesterone caused no recognizable alteration in the turgescent perineum of the baboon.

Experiment 2 (Table 1). Since 0.5 mg. progesterone was inadequate to produce any effect on the turgescent perineum 2 mg. was injected on the 13th day of the cycle. This baboon had a normal menstrual rhythm of 44 days. As in the first experiment no disturbance of the cycle was recognizable and the baboon bled normally on the expected date. In this case 2 mg. of progesterone given in the stage of perineal turgescence were incapable of disturbing the cycle.

TABLE 1

Experiment No.	Length of normal cycle	Day of injection	Dose of progesterone in mg.	Duration of perineal inhibition	Bleeding	Length of experimental cycle
1	38	11	0.5	—	—	38
2	44	13	2.0	—	—	44
3	38	8	5.0	7	—	45
4	44	13	3.0	6	—	53
5	38	10	10	5	—	41
6	42	9	20	5	+	14
7	42	9	15	5	—	45
8	38	5	10	4	—	41

Experiment 3 (Table 1). In this experiment 5 mg. progesterone (10 times the quantity used in experiment 1) were injected into a baboon on the 8th day of the cycle. The normal cycle for this animal was 38 days. The perineum commenced to deturgesce the day after the injection, that is, on the 9th day. This continued until the 13th day when the size of the perineum was 12 inches, 3 inches larger than the normal resting level for this animal (fig. 1). The perineum did not alter its size until the end of the 15th day when it commenced to turgesce once more and from that point proceeded to pass through an almost normal rhythm. No bleeding occurred at the depth of the depression of the cycle caused by progesterone, but bleeding finally occurred on the 45th day, or 7 days later than was expected for this animal.

This experiment showed that 5 mg. of progesterone is adequate to initiate deturgescence in a turgescent perineum produced normally by the baboon's own estrone. The perineum was quiescent for 7 days, a period equivalent to that during which the cycle had been inhibited or depressed by the single dose of progesterone.

Experiment 4 (Table 1). Experiment 3 showed that 5 mg. of progesterone was able to produce perineal deturgescence when given early in the first part of the cycle, but experiment 2 had shown that 2 mg. was insufficient. For this reason a dose of 3 mg. was used to determine whether or not a dose smaller than 5 mg. could produce perineal deturgescence. The animal selected had a normal cycle of 44 days. On the 13th day of the cycle 3 mg. progesterone was injected. Two days later, that is on the 15th day, the perineum commenced to deturgesce and the resting level was reached on the 20th day. Without any evidence of bleeding the perineum commenced to rise on the 23rd day and went through the stages of turgescence, deturgescence and rest. Bleeding occurred on the 53rd day, that is 9 days later than usual. On this occasion postponement of the bleeding was 2 days longer than the period (7 days) during which the turgescence of the perineum had been inhibited by the single dose of progesterone.

From this it is clear that 3 mg. progesterone is adequate to cause perineal deturgescence artificially, when given in the first part of the cycle.

Premature bleeding was precipitated by a single injection of progesterone in experiments 5–7.

Experiment 5. A baboon with a 38-day cycle was injected with 10 mg. progesterone on the 10th day of the cycle. Within 24 hours the perineum underwent rapid deturgescence diminishing in diameter by 2½ inches or one-half its total width. The rate of deturgescence then diminished progressively but the perineum reached its smallest dimensions on the 15th day, 5 days after the injection. By this time the perineum was in a state of rest. On the 16th day the perineum rose rapidly without any evidence (even microscopic) of bleeding and on the 12th day after the injection that is on the 22nd day of the cycle the perineum reached its

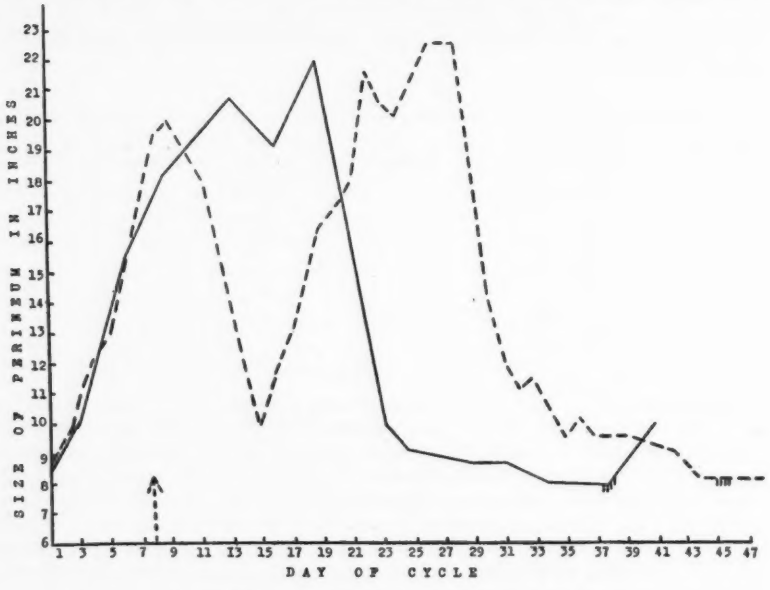

Fig. 1. The effect of 5 mg. progesterone on the perineum of the baboon given on the 8th day of the turgescent stage.

———— normal cycle
– – – – – – – – experimental cycle
//////////////// onset of bleeding

maximum dimensions. The perineum then commenced to deturgesce and bleeding occurred on the 41st day of the cycle. Thus the cycle in this case also was prolonged (3 days).

Experiment 6. As 10 mg. had proved inadequate to produce bleeding the dose was doubled. A baboon with a 42-day cycle was injected with 20 mg. progesterone on the 9th day of the cycle. The perineum underwent an even more rapid deturgescence which was completed on the 13th day. The resting stage was maintained for 1 day and then a bleeding occurred which lasted for 5 days, the normal bleeding period for this animal.

Bleeding had been induced in a normal baboon with 20 mg. progesterone. Bleeding occurred 5 days after the injection that is on the 14th day of the cycle. Thus the normal cycle had been experimentally shortened by no less than 28 days or two-thirds of its ordinary length. The following cycle was shorter than usual, 34 days instead of 42.

Experiment 7. This experiment was designed to determine whether quantities less than 20 mg. could cause bleeding. Experiment 6 had proven that 10 mg. progesterone was inadequate. Another baboon with a 42-day cycle was injected with 15 mg. on the 9th day of the

cycle. The perineum diminished its size by 4 inches in 24 hours, reached its resting level on the 13th day and then without any evidence of bleeding, commenced to rise on the 15th day and proceeded in a normal fashion to continue its cycle.

Thus 20 mg. progesterone causes bleeding whereas 15 mg. does not. The quantity of progesterone necessary to cause premature bleeding in the adult female baboon is somewhere in the neighborhood of over 15 mg. and not more than 20 mg.

As in these experiments it had not always been possible to inject the progesterone in each case on the same day of the cycle, the possibility had not been excluded that, by giving the same dose of progesterone on different days of the cycle, the effects might be different. For this reason another experiment was performed.

Experiment 8. This baboon had been used previously in experiment 5 where 10 mg. had been injected on the 10th day of the cycle. After 2 cycles had intervened and normality of

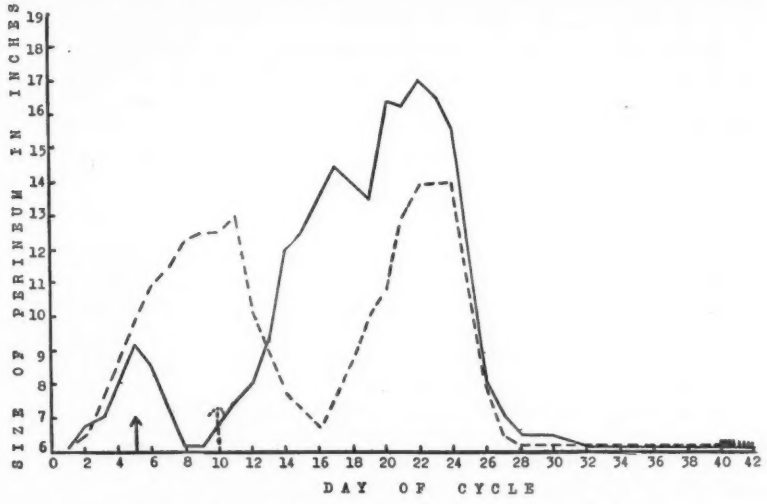

Fig. 2. The effect of 10 mg. progesterone on the perineum of the baboon given in the turgescent stage.

——————— injected on day 5 of the cycle
– – – – – – – – injected on day 10 of the cycle
,,,,,,,,,,,,,,,,,,, bleeding

cyclical phenomena had been well established the same baboon was subjected to the administration of 10 mg. progesterone on the 5th day of the cycle. The perineum again underwent deturgescence within 24 hours after the injection (fig. 2) and continued to diminish in size until the 9th day. Thus a day less was now consumed in arriving at the resting stage. Then, without showing any evidence of bleeding, turgescence commenced once more on the 10th day and the subsequent course of the cycle was normal. Menstruation occurred on the 41st day that is the cycle was prolonged by 3 days, the identical number of days it had been prolonged when previously interrupted on the 10th day.

The administration of 'one-half the bleeding dose' of progesterone on different days in the first part of the cycle affects slightly the length of the cycle, but to a relatively equivalent extent irrespective of the day of injection. A point of particular importance too is the stability of the results in the same experimental animal, thus demonstrating the reliance that can be placed on the time factors involved in all these experiments.

DISCUSSION

A previous study (1) proved that progesterone given repeatedly in the first or

turgescent part of the cycle, causes deturgescence within 24–48 hours after the initial injection.

The present series of experiments shows that 3 mg. of progesterone given in the first part of the cycle is adequate to produce a perineal deturgescence lasting for 7 days (experiment 3). Quantities less than 3 mg. produce no recognizable changes in the perineum. It is an 'all or none' reaction!

Turgescence of the perineum in the baboon is a positive phenomenon caused by estrone (4, 5, 6). Deturgescence of the perineum in normal baboons going through ovulatory cycles is an equally positive phenomenon induced by progesterone (1). If the deturgescence were produced through an inactivation of estrone by progesterone, as Corner (7) seems to think, then 3 mg. of progesterone is adequate to inactivate the amount of estrone which is maintaining the baboon's perineum at its customary size on the 8th day of the cycle.

The question then naturally arises as to whether the 3 mg. progesterone is actually inactivating the estrone and so lowering the 'estrone threshold' to such an extent as to cause perineal deturgescence or whether progesterone is exerting a positive deturgescent effect in the presence of and despite large quantities of estrone? In the latter event the resulting effect, whether deturgescence or turgescence would depend rather on the qualitative advantage of one hormone over the other. Before progesterone can exert its effect both on the endometrium and sexual skin preliminary sensitization with estrone is indispensable.

Progesterone and bleeding. Repeated administration of progesterone in total quantities of 15 mg. or more in the first part of the cycle of the normal baboon results in premature bleeding when the progesterone is withheld (1). Experiments 5, 6, 7 have shown that it is possible to precipitate bleeding by giving a single dose of 20 mg. of progesterone. The perineum itself rapidly undergoes deturgescence and bleeding occurs on the 5th day after the injection.

In earlier experiments with multiple injections it has been shown that, provided the total quantity of progesterone given was 15 mg. or more, bleeding always followed the withdrawal of progesterone. This bleeding could be provoked by giving 3 injections of 5 mg. or 2 injections of 7.5 mg. and the time of onset of bleeding after withholding progesterone was 144 to 120 hours respectively. If the total doses of progesterone were larger than that then the bleeding occurred much earlier after withholding progesterone, the earliest bleeding thus far obtained by this method being 68 hours after a total dosage of 51 mg.

On comparing the dosage required to cause bleeding by giving multiple injections and single injections it is evident that a smaller quantity is required by the former method than by the latter. It has been possible to cause bleeding with a total quantity of 15 mg. given as 2 or 3 injections whereas 15 mg. was inadequate when given in one dose and only 20 mg. could cause bleeding. In those instances where two or three doses of progesterone are given at fixed intervals there is probably a more prolonged or 'staircase' type of action on those structures responsible for bleeding than when only a single dose of progesterone is used.

With a dosage of 20 mg. bleeding occurred 5 days after progesterone withdrawal. On the day after the onset of bleeding, turgescence is re-initiated. This means that coincidentally with, or 24 hours after bleeding estrone is being poured into the circulation in sufficient quantities to re-initiate perineal turgescence.

It may of course be that the onset of bleeding is the culminating effect of progesterone and that sufficient estrone to cause turgescence was available all the time but was unable to act in the presence of progesterone.

On the other hand Corner (7) makes the assumption that progesterone in some way "suppresses the action of estrogenic hormone upon the endometrium while itself preventing uterine breakdown, so that when progesterone is discontinued the animal is then deprived of both hormones."

But it must be noted that estrone given in large quantities in the second half of the cycle of a normal baboon cannot cause turgescence in a deturgescent perineum and thus prevent bleeding (6). Bleeding supervened despite the presence of large quantities of estrone in the circulation. Hisaw and Greep (8) have found that the presence of progesterone makes it difficult to inhibit bleeding by subsequent injection of estrogen even when quantities are used which normally can prevent bleeding in castrated animals. According to Hisaw and Greep progesterone therefore modifies the endometrial stroma even in the presence of large quantities of estrogen.

In the prepubertal baboon the withdrawl of estrone after 10 to 15 days treatment is followed by bleeding on the 10th to 16th days (5); but if one or two injections of progesterone are given on the day estrone is withdrawn bleeding is precipitated on the 5th or 6th day after progesterone administration that is 5 to 11 days earlier than would be expected if estrone alone were used. Hence the changes in the endometrial stroma preliminary to bleeding are rapidly expedited by the presence of progesterone, although bleeding would have occurred ultimately even if no progesterone were employed. It seems that the presence of estrone at an early stage in the cycle is necessary if bleeding is to follow, whether progesterone is used or not.

In a previous paper (9) it has been shown that 1½ to 2 mg. of progesterone is necessary to inactivate the effect of 1 mg. of estradiol benzoate on the perineum of prepubertal baboons when given simultaneously, after a preliminary course of estrone treatment for 10 days. But when 3 mg. of progesterone is given simultaneously with 1 mg. estradiol benzoate from the outset and without any preliminary estrone medication the perineum swells, though not to the same extent as with estrone alone. Even when 5 mg. of progesterone is given simultaneously with 1 mg. estradiol benzoate it is not possible to completely prevent perineal deturgescence (10). These experiments on the prepubertal baboon show very forcibly, first that before progesterone can exert its action on uterus and perineum these organs must be sensitized by estrone; secondly that progesterone can exert its action in the presence of large quantities of estrone.

From this it follows that the action of progesterone does not depend primarily on its ability to inactivate or directly suppress estrone, but rather that progesterone can act on the uterus and perineum in the presence of large quantities of estrone however little they have been sensitized previously by estrone.

In normal baboons therefore, two responses can be elicited by giving progesterone in single doses in the first part of the cycle, namely, perineal deturgescence without bleeding and perineal deturgescence with bleeding.

If the dose of progesterone is as little as 3 mg. and less than 20 mg. the perineum will undergo deturgescence for 4 to 7 days and then will commence to turgesce again. If the dose is 20 mg. or more deturgescence sets in and at the end of the 5th day bleeding supervenes. The 'bleeding dose,' when given as a single injection, is about 6 times greater than the dose necessary to cause deturgescence alone. If Corner's assumption is correct, then the amount of estrone inactivated by the 'bleeding dose' in the baboon is equivalent to 15 mg. estradiol benzoate, which in turn is equivalent to 150,000 international benzoate units. This appears an incredible amount of estrogen to be circulating in the blood at any particular moment.

This dual effect of progesterone of causing deturgescence and of provoking bleed-

ing in the baboon is of great value since it is now possible to assay progesterone with very great accuracy for 3 mg., 15 mg., and 20 mg. quantities. The first or 3 mg. quantity affects the perineum only; the second, or 15 mg. quantity when given in 2 or 3 doses at 3 to 4 day intervals causes deturgescence and bleeding 120 to 144 hours after the last injection; the third, or 20 mg. quantity, used as a single dose, produces deturgescence and precipitates bleeding 120 hours later. Reversing our approach and giving progesterone in doses adequate to produce bleeding it is possible to determine whether estrone is present even when in minute amounts, for bleeding will not occur if estrone is absent.

The latent period preceding bleeding. After progesterone administration there is always a period of 4 to 7 days, which intervenes between the injection and the final reaction whether bleeding occurs or not. This delay is not entirely due to a slow absorption of progesterone since the external effect on the perineum is noticeable 6 to 24 hours after the injection. This latent period has occurred in all experimental animals where progesterone was used.

This phenomenon has also been described for castrated *Macacus rhesus* (11). It was found that, after a preliminary course of estrone treatment followed by progesterone, bleeding occurred on the 5th day after progesterone withdrawal. The latent period in the normal baboon is reduced greatly in length after the combined administration of estrone and progesterone, but given successively in the order mentioned than when estrone is given alone (vide infra).

The effect of sub-bleeding doses of progesterone on the course of the experimental cycle. In those cases (experiment 1 and 2) where progesterone is insufficient to cause any change in the perineum the cycle is unaffected and menstruation occurred at the expected time. This again confirms the close relation existing between the changes in the perineum and the menstrual cycle. On the other hand, where the dose of progesterone is adequate to cause perineal deturgescence the length of the menstrual cycle has been affected in all cases. The cycle has been consistently lengthened by 3 to 9 days. Where multiple injections have been given in quantities insufficient to cause bleeding the cycle has been lengthened approximately by the length of the induced deturgescence. In the case of the single injections the cycle, although lengthened, is not prolonged by the same extent as the experimentally induced deturgescence. Only in experiment 4 was the cycle lengthened by a period longer than the period of induced deturgescence.

Where sufficient progesterone is given to cause bleeding the cycle is shortened. In experiment 6 the cycle has been shortened by two-thirds its normal length.

The lengthening of the experimental menstrual cycle by single doses of progesterone is not as constant a phenomenon as after multiple injections of progesterone.

SUMMARY

The normal adult female baboon, when given progesterone in the first or turgescent part of the cycle, exhibits specific perineal reactions which may or may not be accompanied by bleeding at the end of 4 to 7 days after progesterone administration.

The perineal reaction consists of a sudden onset of deturgescence when the sex skin may become completely absorbed. The minimum quantity of progesterone necessary to produce this effect is 3 mg.

Bleeding occurs if a single dose of 20 mg. progesterone is used and is precipitated on the 5th day after the injection when the perineal swelling has reached its lowest ebb. Doses smaller than 20 mg. produce perineal deturgescence unaccompanied by

bleeding. If 2 or 3 injections are given at 3 or 4 day intervals then a total of 15 mg. of progesterone is adequate to produce bleeding. Progesterone withdrawal is capable of precipitating bleeding and in so doing the normal cycle of a baboon can be shortened to one-third its normal length.

Where bleeding is not produced by the single administration of progesterone the cycle is prolonged by 3 to 7 days as a rule. The administration of appreciably large quantities of progesterone (10 mg.) early or late in the first part of the cycle has no effect on the bleeding.

A minimal amount of progesterone is necessary to produce perineal deturgescence without bleeding and perineal deturgescence with bleeding. Progesterone acts in the presence of large quantities of estrone and the action of progesterone is not fully explained by a quantitative inactivation of estrone. In the baboon the uterus and perineum, after they have been sensitized to estrone, have a greater affinity for progesterone than for estrone and progesterone is active in the presence of large quantities of estrone.

Two reactions of the baboon, perineal deturgescence without bleeding and perineal deturgescence with bleeding permit an easy and accurate assay of progesterone uncomplicated by operative procedures.

I wish to acknowledge my indebtedness to Professor Raymond A. Dart for his encouragement and advice throughout the course of this investigation. My sincere thanks are also due to Dr. H. B. Stein for his assistance and advice in planning the experiments. Messrs. Schering Corp. have generously supplied the progesterone (proluton) used in all the experiments.

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THE POTENCY OF CERTAIN COMMERCIAL HORMONE PREPARATIONS. SECOND STUDY

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Because of the interest shown by many physicians in a previous report[1] on the same subject, it seemed desirable to determine again the biologic potency of a number of commercial hormone preparations. Several years have elapsed since the original assays were made, during which time endocrine therapy has become even more popular, a number of new products have been introduced and some changes in methods of preparation and administration of older products have been made. The author wishes to emphasize again that the purpose of this study is not to 'show up' unsatisfactory products, but to indicate that the number of 'units' on a given vial may not be a reliable indication of biologic potency unless the question of assay method and possible deterioration is taken into account.

It was, of course, impossible to study all commercial hormone preparations on the market, those included represent the ones having the widest sale in this region. As in the previous study, all were purchased across the counter, as the clinician would purchase them, from two of Denver's largest and most reputable prescription pharmacies. The hormones included are estrogens, corpus luteum preparations, gonadotropins from pregnancy urine, gonadotropins from the anterior lobe, growth hormones and adrenal cortex hormones.

TABLE 1

Preparation	Number of I.U. equivalent to 1 R.U.
International Standard	11.0
Reed and Carnrick, Estrogenic Hormone #720	7.5
Roche-Organon, Menformon #309138	12.5
Endo Products, Inc., Estromone, #9476	10.0
Lakeside Laboratories, Estrolin, #9922	8.5
Squibb, Amniotin #61982	13.5
Parke, Davis, Theelin #3193908	9.0
Schering, Progynon-B 6-K-09	7.5
Abbott, Estrone #3578	11.0

Estrogens in oil, for parenteral use. All products in this group have their potency indicated in International Units. The assay was made at 4 dosage levels, 5, 10, 15 and 20 I.U., 24 animals being used on each dose. The results were plotted and the point at which 50% of the animals showed full estrous smears taken as the rat unit. They were assayed directly against the international standard material, supplied through the kindness of Dr. Parkes, of the National Institute for Medical Research. The results are given in table 1.

It may be concluded that all of these materials are stable, well assayed and contain the labelled content. Differences between them are probably of no significance and merely reflect inaccuracies in the assay.

[1] D'AMOUR, F. E., AND M. C. D'AMOUR: *Endocrinology* 22: 583. 1938.

Estrogens in aqueous solution. The method was the same as for the previous group except that the standard was administered suspended in a 10% alcohol solution. The results are given in table 2.

TABLE 2

Preparation	Results
International Standard	43 I.U.—1 R.U.
Lakeside Laboratories, Estrolin #9902	400 I.U.—1 R.U.
Parke, Davis, Theelin #3062794, (stated to contain 50 R.U. per cc.)	½ cc.—0% positive 1 cc.—17% positive

Two aqueous estrin preparations assayed in the study previously published did not contain nearly the amount of biologic activity stated by the label to be present. One of these (the Schering product) has since been withdrawn. The same lack of potency is shown above. It seems definitely established that aqueous estrin preparations are unsatisfactory.[2]

Estrogens for oral administration. Although intended for oral use, the following 5 preparations, with the exception of emmenin, are assayed parenterally by the makers. We were concerned primarily with the question of labelled potency; however, since oral administration seems to be increasing in popularity, the assays were repeated, giving the material by stomach tube. Since the results are not readily tabulated, each preparation will be discussed individually.

(*a*) Schering, Progynon-DH, 8381. This preparation comes in tablet form. The stated potency is 200 "Active Biological Units" per tablet. Our assay results were, single injection, suspended in oil, 533 R.U. per tablet; orally, single administration, suspended in saline, 20 R.U. per tablet.

(*b*) Squibb, Amniotin for Oral Administration, 58680. This preparation comes in oil-filled capsules. The stated potency is 2000 I.U. per capsule. Our assay results were single injection, in oil, 11.0 I.U. = 1 R.U., (182 R.U. per capsule); orally, single administration, in oil, 2000 I.U. = 1 R.U. (1 R.U. per capsule).

(*c*) Parke, Davis, Theelol, 3175451. This preparation comes in a powder-filled capsule. The stated potency is 2000 vaginal canalization units (0.12 mg.) per capsule. Our assay results were, given by injection in divided doses, (twice daily for 3 days), 1 labelled U = 30%, 2.5 U = 50%, 5 U = 80% positive; in single dose, 10 U = 0% positive; orally, in divided doses, 10 U = 30%, 20 U = 100% positive; in single dose, 100 U = 0% positive.

(*d*) Abbott, Estriol, 75493. This preparation resembles the previous one. Our assay results were, given by injection in divided dose, 1 labelled U = 50% positive, 2.5 U = 80% positive, 5 U = 100% positive; in single dose, 10 U = 0% positive; orally, in divided dose, 10 U = 50%, 20 U = 100% positive; in single dose, 100 U = 0% positive.

(*e*) Ayerst, Emmenin, R8049. This comes in liquid form. The stated potency is 1 cc. = 30 'day-oral' units. Our assay results were, given in drinking water for 3 days, 3 'day-oral' U = 80% positive.

Repetition of the makers' assay showed all 5 to contain approximately the labelled number of units. Since the methods of assay differ widely, direct comparison is impossible. It must be remembered that the amount of tri-hydroxy estrin (the form present in the last 3 preparations) necessary to produce cornification in adult castrate females is hundreds of times larger than that required in immature rats. Adult castrates are used for the first 2 preparations, immature rats for the last 3. The unit used to express the potency of the last 3 preparations is therefore very small to begin with. Further, the divided dosage technic is employed, giving a still smaller unit. It seems unfortunate that this small unit is further reduced (to one-third) by the introduction of the 'day-oral' unit in the case of emmenin. As regards oral administration of the

[2] Since this paper was prepared, the Lakeside Laboratories have also withdrawn their aqueous estrin preparation from the market.

first 4 preparations, it will be seen that the potency by that route of administration is very low. We consider it highly desirable that preparations intended for oral use be assayed orally.

Corpus luteum hormone. This assay depends upon the development of progestational proliferation of the uterine endometrium of the rabbit. The standard for comparison is the 8-day pregnant uterus and a comparable reaction is given (in the Corner-Allen method) by 1 mg. crystalline progesterone. This is the international unit. In our assay, this reaction was given a rating of 4, and the experimental uteri were given comparable ratings, 1 representing a minimal response. The test is not entirely objective, depending somewhat on the operators' judgment. Three rabbits were used for each assay and sections of all uteri were prepared. The results are given in table 3.

TABLE 3

Preparation	Makers' Assay	Our Assay
Parke, Davis, Lipo-Lutin 3216450	1 I.U. per cc.	4·4·3
Lakeside Laboratories, Progestin 7110	1 Corner-Allen unit per cc.	4·4·3
Upjohn, Progestin 15405	1 Corner unit per cc.	4·4·2
Schering, Proluton 8-ED-14	1 mg. cryst. progesterone per cc.	4·4·2
Lilly, Corpus Luteum Extract, 225-910220	0.2 gm. desiccated corpus luteum per cc.	2·1·1
Hynson, Westcott and Dunning, Lutein Solution	Water-soluble extractive of 0.2 gm. dried substance	1·1·1

The first 4 preparations contain the labelled potency, minor variations in the assay are of no significance. It is perhaps not surprising that the last 2 should give such poor results as they are apparently not biologically assayed and are in aqueous solution. The deterioration noted in aqueous estrin preparations seems to apply also to progestin.

TABLE 4

Material	Makers' Assay	Our Assay	
		Dosage	% Positive
Lakeside Labs., APL Sex Hor. Conc. 9400. See *A* below	100 R.U./cc. Foll. and ut. stim'n, lut'z'n.	1 R.U.	40
		2 R.U.	90
		2 R.U.	0
		5 R.U.	60
Endo Products, Entromone 10307. See *B* below	125 R.U./cc. No method given	1 R.U.	0
		5 R.U.	10
		10 R.U.	50
Parke, Davis, Antuitrin-S 3208021	100 R.U./cc. lut'z'n	1 R.U.	70
Squibb, Follutein 63638	100 R.U./cc. lut'z'n	1 R.U.	70
Ayerst, APL 7951. See *C* below	500 Collip R.U./cc. Vag'. Est. in 50%	3 Collip R.U.	50
Winthrop Chem., Antophysin, 8625. See *D* below	100 R.U./cc. Vag'l smear, hyper. of ut., large Foll. and C.L.	1 R.U.	70

A. Expiration date, 4/19/39. First 2 assays were made 10/29/38, last two, 4/12/39. A considerable loss of activity occurred, although material was kept in the refrigerator.

B. Expiration date, 1/7/39. Assay completed 12/10/38. On 5 U 1 vagina was open and uterus slightly dilated, on 10 U the same was true of 5 animals. The ovaries were negative on all doses.

C. Expiration date, 1/15/39. Assay completed 10/15/38.

D. Vaginal estrus in 2, uterine dilation in 7, follicular response in 7, luteinization in 1.

Gonadotropic hormones. The only generalization which can be made concerning assay methods of these substances is that hardly any two of them are alike. This emphasizes the importance of the fact that an international standard for those derived from the urine of pregnancy has been established. A study of the international standard is reported in the following paper in this journal. The establishment of such a

standard does not, however, entirely solve the problem, as is shown in the case of aqueous estrin solutions and in the case of estrogens for oral administration, where much confusion still exists. The results of the present assay cannot be used to make comparisons of activity but indicate only how nearly the labelled potency agrees with that found by us. The results, for APL preparations, are given in table 4 and those for gland products in table 5. Without exception 10 animals were used on every dose.

The lack of uniformity in assay methods, which makes comparison of potencies quite impossible, has already been stressed. Where one maker uses 21-day rats, an-

TABLE 5

Material	Makers' Assay	Our Assay	
		Dosage	% Positive
Searle, Gynantrin, 61771	100 R.U./cc. Foll. form'n and lut'z'n	1 R.U.	0
		5 R.U.	0
		10 R.U.	0
Chappel, Prephysin 140-P and 146-P. See *A* below	25 R.U./cc. 50–100% increase in wt. of ovaries	1 R.U.	0
		2.5 R.U.	0
		5 R.U.	80
Parke, Davis, Antuitrin 3099247. See *B* below	No claims made	1 cc.	0
Armour, Gonadotropic factor, 020128. See *C* below	300 Collip units/cc. Ovaries weigh'g 35 mg.	30 Collip U.	0
		150 Collip U.	10
		300 Collip U.	80
Ayerst, Ant. Hypo. G.T. Factor R8432. See *D* below	300 Collip units/cc. Ovaries weigh'g 35 mg.	30 Collip U.	30
		60 Collip U.	60
		60 Collip U.	60

A. Weight of control ovaries was 10.2 mg. Demanding a 50% increase, a weight of 15.3 mg. is considered positive. Average weight on 1 U.=10.7 mg., on 2.5 U.=12.8 mg., on 5 U.=20.9 mg., with 8 weighing over 15.3 mg.

B. No claims for activity are made. One cc. per rat, (using 30-day animals and divided doses) gave ovaries averaging 12.0 mg., as compared with control weight of 10.2 mg. No vaginal or uterine response was seen.

C. Thirty Collip units=average weight of 20.4 mg., 150 U.=25.4 mg. (1 over 35 mg.) and 300 U.=42.4 mg. average, (8 over 35 mg.).

D. Expiration date, 6/15/39. First 2 assays were made 11/15/38, the last, 5/22/39. On the first assay 30 Collip units gave ovaries averaging 31.0 mg. (3 over 35 mg.), on 60 U. the average was 38.2 mg. (6 over 35 mg.). The last assay on 60 U. gave ovaries averaging 40.3 mg. (6 over 35 mg.). There was no deterioration on standing 6 months in the refrigerator.

other 30-day rats, another vaginal estrus as the endpoint, another increase in weight of ovaries, another luteinization, etc., it is all too likely that the clinician, amid this welter of confusion, will be guided mainly by the number of units stated on the label to be present. The one uniform factor so far has been that the 'unit,' whatever else it may have been, has always represented the total dose. Now, however, even this is no longer true. There appears to be no justification for dividing the 'unit' into thirds and thirtieths, as is done by the introduction of the Collip unit. Nothing could be more confusing than to have the Collip unit, in the case of APL, represent the daily dose, (i.e., one-third the usual unit) and in the case of gland preparations, represent one-tenth the daily dose (i.e., one-thirtieth the usual unit).

Growth hormone. The 'growth unit' used by the makers of these products is the amount given per day, over a 10-day period, which will produce a 10% increase in

weight of adult female rats weighing approximately 250 gm. Four products, from 2 manufacturers, were assayed. The results are given in table 6.

TABLE 6

Material	Makers' Assay	Our Assay	
		Dose	Results
Squibb, Ant. Pit'y Ext. 63254	10 Growth units/cc.	0.1 cc. daily	0.5% increase
Squibb, Ant. Pit'y Ext. 63254 and 62891	10 Growth units/cc.	0.3 cc. daily	2.3% increase
Parke, Davis, Antuitrin Growth, 3210491	10 Growth units/cc.	0.1 cc. daily	0 % increase
Parke, Davis, Antuitrin Growth, 3210491 and 3219393	10 Growth units/cc.	0.3 cc. daily	4.7% increase

These preparations contain considerably less than the labelled potency.

Adrenal cortex preparations. These were assayed by determining their ability to maintain the life of adrenalectomized rats. The problem of assaying cortical hormone preparations has been discussed in the paper previously mentioned.[1] The results are given in table 7.

TABLE 7

Material	Makers' Assay	Our Assay
Armour, Suprarenal Cortex 526076	3 gr. tablets	No activity in 2 tablets daily
Armour, Suprarenal Cortex Liquid, 602112	250%	No activity in 1 cc. daily
Roche-Organon, Cortin. No control numbers	At least 1 provisional rat unit per cc.	2 R.U. per cc.
Wilson, Adrenal Cortex Extract, 569 and 578	Each cc. derived from 75 gm. suprarenal tissue	4 R.U. per cc.
Parke, Davis, Eschatin, 3201860	1 cc.=25 dog units	4 R.U. per cc.
Schiefflelin, Cortisorbate, 808452 and 970830	1 R.U. per tablet	1 R.U. per tablet

The rat unit used in our assay represents the daily amount necessary to keep young, adrenalectomized rats alive and healthy for a 10-day period. On this basis, the Armour products were inactive in the doses given, however, no claims are made for its activity nor is it recommended by the makers for use in Addison's disease. The last 4 products are potent and contain the expected activity.

SUMMARY

The following conclusions concerning the preparations studied are reached on the basis of the assays described.

Estrogens in oil, for parenteral use, are uniformly potent. Aqueous estrogens apparently deteriorate and do not contain the labelled potency. A great deal of confusion exists in the assay of estrogens for oral use. They should be assayed orally. The progestin preparations in oil are uniformly potent, aqueous ones are not.

Much confusion exists in the assay of gonadotropic substances. This will, to some extent, be eliminated by the use of International Standard Gonadotropin, for pregnancy urine derivatives. Problems of deterioration, possible oral administration, etc., will not be overcome, nor does this standard apply to other gonadotropic agents. Efforts to decrease the size of units by the introduction of fractions is to be decried.

The growth hormones apparently deteriorate on standing, they do not contain the labelled potency.

Most of the cortin preparations studied are potent and contain the expected activity.

THE BIOLOGIC POTENCY OF INTERNATIONAL STANDARD CHORIONIC GONADOTROPIN[1]

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THE LEAGUE OF NATIONS HEALTH ORGANIZATION has recently announced (M. 40, —April, 1939) the establishment of an International Standard for the gonadotropic substance of human urine of pregnancy: chorionic gonadotropin. The standard material represents a pool from 6 sources, diluted with lactose and made up in tablets of approximately 1 mg. each. The unit is defined as follows. "The specific gonadotrophic activity of 0.1 mg. (100 gamma) of the standard preparation shall be the International Unit for recording the activities of all gonadotrophic preparations of human urine of pregnancy, *but only of such.*"

Recommendations for the use of the standard in biologic assays, taken from the Report of the Conference, Bulletin of the Health Organization of the League of Nations, 7, 889, 1938, are as follows.

The only tests for the comparative determination of gonadotrophic activity, in units as above defined, on which the Conference has evidence to justify recommendation, depend on:

a. The observation of a direct or indirect gonadotrophic effect, shown by morphological changes in the gonads:

b. The observation of secondary changes in the accessory reproductive organs, in animals not deprived of their gonads. When this type of test is used, the absence of substances directly causing such changes in the accessory reproductive organs should be assured by control tests on animals deprived of their gonads.

We have recently published (1) the results of a comparison of various biologic methods used in the assay of gonadotropic substances. In view of the wide use which will be made of the International Standard material, it seemed desirable to make a similar study of this material, for the purpose of *a*), Determining its biologic potency as indicated by different methods of assay, and *b*), Determining which of these methods, according to established criteria, seemed best suited to commercial assay application. A supply of the standard was kindly supplied by Dr. Parkes, of the National Institute for Medical Research.

EXPERIMENTAL

Five methods of assay were used: (*a*) increase in weight of seminal vesicles; (*b*) opening of vagina and production of vaginal cornification; (*c*) increase in weight of ovaries; (*d*) increase in weight of uteri (method of Levin and Tyndale), and (*e*) production of corpora lutea. The rats were 21 days old at the time of first injection, except that one group of 30-day animals was used in the corpus luteum test, for the reason that animals of this age are frequently used by commercial producers. The solutions were made up fresh, in physiologic saline, for each series of injections, injections were

[1] This investigation was aided, in part, by a grant from the National Research Council, Committee on Endocrinology.

made once each day for 3 days and the animals killed at the end of 100 hours. Each point on the curves represents, for the males, 20 animals, for the females, 10 animals. All organs were cleanly dissected using a binocular loup, immersed in saline as soon as removed, blotted on filter paper and weighed on a chainomatic balance. Inspection for corpora lutea was made macroscopically.

RESULTS

The results are given in table 1. For convenience in making certain comparisons the seminal vesicle and ovarian weight increases are plotted in figure 1 and the uterine weight and vaginal smear reactions in figure 2. It will be noted that the international unit, 100γ doubles the size of seminal vesicles, gives 100% positive estrous smears, yields nearly a maximum increase in uterine weight, has little effect on ovarian weight, gives no luteinization in 21-day-old rats and gives 30% luteinization in 30-day-old rats.

TABLE 1

Males		Females					
Dose	Wt. S.V.	Dose	Ov. Wt.	Ut. Wt.	Est. Smear	C. L. 21 days	C. L. 30 days
γ	mg.	γ	mg.	mg.			
0	6.1	0	11.9	15.0	0	0	0
50	8.3	25	—	15.6	0	0	0
75	9.0	50	13.2	79.8	80%	0	0
100	13.7	100	—	96.1	100%	0	30%
125	13.9	150	14.6	99.4		0	60%
150	15.0	300	16.3	112.8		0	90%
200	19.3	1000	22.0	100.2		70%	100%
400	21.5	1500	22.6	—		90%	
800	21.7	3000	25.7	—		100%	
1500	25.9	6000	31.7	—			

DISCUSSION

To be satisfactory, an assay method should be objective, sensitive, allow for individual variation and be as simple as possible. Applying these criteria to the methods used, the authors have reached the following conclusions.

(*a*) To allow for individual variation, at least 10 animals per dose must be used, regardless of the method.

(*b*) The corpus luteum method should be definitely ruled out of consideration. If macroscopic examination is relied on, it fails on the score of objectivity, if microscopic sections are made, which, in our belief is absolutely necessary to be sure of the degree of luteinization, it fails on the score of simplicity.

(*c*) The ovarian weight method lacks sensitivity; it will be noted that the curve rises very slowly, more than 10 I.U. being required to double the ovarian weight. Excessively large amounts of material must therefore be used. It is reasonably simple, although dissecting out the ovaries is time-consuming.

(*d*) The seminal vesicle method is satisfactory on all counts. In our colony, 1 I.U. almost exactly doubles the seminal vesicle weight.

(*e*) Vaginal cornification and uterine weight methods are considered together, because, as it seems to us, they might well be employed together, the first being used as a check on the second. Figure 2 indicates how closely the responses parallel each other. The advantages are as follows. The same animals are used for both tests. What the vaginal cornification test lacks in objectivity, i.e., in interpretation of the smears, is balanced by the objectivity of uterine weights. Both tests are extremely sensitive,

both require only small amounts of material. The absolute weights of the uteri are great, making great exactness in weighing less important and the increase in weight over the controls is also great, being 6-fold for the uteri as compared to only 2-fold for the seminal vesicles, or one international unit. Removal of the uteri, stripping them of fluid and weighing, is at least as simple as dissecting out the seminal vesicles.

One disadvantage of the method, however, must be stressed. The possible estrin content of the preparation being assayed must be taken into account, as estrin will,

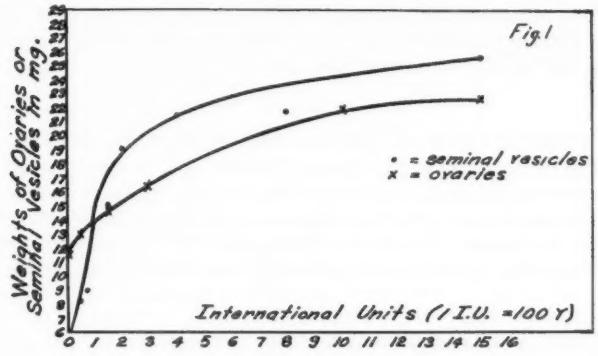
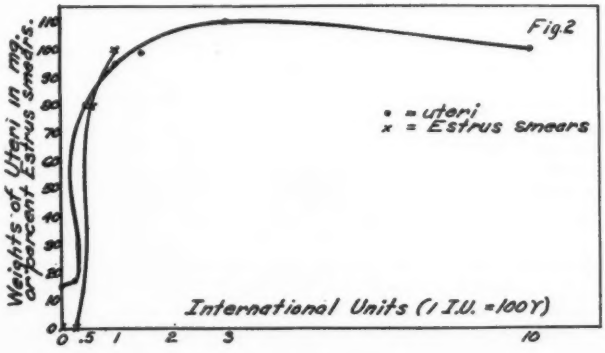


Fig. 1
Weights of Ovaries or Seminal Vesicles in mg.
• = seminal vesicles
x = ovaries
International Units (1 I.U. = 100 γ)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



of course, give vaginal cornification and uterine development in the intact animal. This is the point which the Conference no doubt had in mind in formulating the second paragraph of the recommendations quoted. Injection of 3,000γ, (30 I.U.) into castrate females gave no indication of estrus, it is therefore certain that this material contains no estrogenic substance. The same fact must always be established for each preparation assayed against it. However, most laboratories maintain a colony of castrate females and the additional work entailed in demonstrating the absence of estrin would not be great.

In deciding between the seminal vesicle and the uterine weight-vaginal cornification methods another factor must be considered. In time, no doubt, an international standard for anterior lobe preparations will be established and confusion will be avoided if the same assay method could be used for it as well. The seminal vesicle test is not satisfactory for anterior lobe preparations because of the slight response,

while the uterine weight method as reported by Heller, et al. (2), would appear to be very good, at least for follicle-stimulating fractions.

Levin and Tyndale (3), in describing the uterine weight method as developed in the mouse, point out that it is less accurate for pregnancy urine and pregnant mare serum gonadotropins than for those present in castrate urine, as there is more individual variation on minimal doses, although with higher doses the response is more uniform. We believe that the use of a sufficiently large number of animals and the performance of the assay at dosage levels sufficiently high, would overcome the objection of individual variation, which, after all, is inherent in all biologic assays. Everything considered, the uterine weight-vaginal cornification method seems to us the one best suited in all respects to routine assay use.

SUMMARY

The International Standard Chorionic Gonadotropin has been assayed by 5 methods. The biologic potency of the international unit, 100γ of this preparation, has been determined. Evaluation of the data leads to the conclusion that the combination of uterine weight and vaginal cornification methods, the absence of estrin having been assured, represents the most desirable method for the use of this material in the assay of gonadotropic substance originating from human pregnancy urine.

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THE INFLUENCE OF VARIOUS PHYSIOLOGICAL SUBSTANCES ON THE GLYCOGENOLYSIS OF SURVIVING RAT LIVER[1]

THE INFLUENCE OF CORTICAL HORMONE ADDED IN VITRO

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In two preceding communications (1, 2) the in vitro influence of bile salts and insulin on the glycogenolysis of surviving rat liver slices has been investigated with the view of explaining the pathogenesis of Von Gierke's glycogen disease. The next hormone to be considered in this series of experiments was the cortical hormone of the adrenals. It has been demonstrated during the last decade that the adrenal cortex plays an important part in regulating carbohydrate metabolism in mammals. Whereas these studies have been made in vivo, it is the purpose of the present paper to examine the direct in vitro influence of cortical hormone on the process of hepatic glycogenolysis in surviving tissue slices.

METHODS

The methods applied were essentially the same as those described in the first and second communications (1, 2). Liver slices were prepared from non-fasted male rats 5 to 8 months of age with only 2 female animals in the series. The time of the experiments was from December to April, between 9 and 11 A.M. Six or eight 50 cc. Erlenmeyer flasks containing 0.23 to 0.29 gm. of sliced liver tissue in 5 cc. of suspension solution were used in each experiment. Except for the hormonal extract added, the suspension solution consisted of a phosphate-buffered physiologic salt solution of pH 7.4 (37° C). Starting at a 'zero' time 20 to 28 minutes after the animal's death, glycogen was determined in the unsliced zero sample and at various intervals in the liver slices (4 to 5 duplicate determinations in each experiment). The methods used were those of Good, Kramer and Somogyi (3) for glycogen precipitation and of Somogyi (4) (Shaffer and Hartmann) for glucose estimation. For further details of the technic see the two preceding communications (1, 2).

The cortical hormone used in these experiments was a preparation called eschatin supplied without preservative by Parke, Davis & Co.[2] Eschatin is a highly purified extract of the adrenal cortex manufactured according to the Swingle and Pfiffner method (4a). Dissolved in an aqueous solution of 0.9% sodium chloride, the preparation contains 1 to 2 mg. of gland extracts per 1 cc. corresponding to 40 gm. of fresh whole beef adrenals. Besides the active principle some related steroids are present in the extract. It assays approximately 50 dog units or 4 rat units per cc. The adrenalin content is less than one part per million. The vials were kept at refrigerator temperature during the experimental period, but not for more than 5 weeks per sample. The dosage applied on an empirical basis in the in vitro experiments varied between 0.5 and 2 cc., or 2 and 8 R.U., per 5 cc. of suspension solution.

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[1] This work was supported by a grant from the Douglas Smith Foundation for Medical Research.

[2] The writer wishes to thank Dr. Oliver Kamm of the Parke, Davis Laboratories for supplying this special extract along with detailed information upon it.

EXPERIMENTAL RESULTS

Controls. Since eschatin reacts approximately neutral no change in pH was seen in the suspension solution after its addition. The extract did not contain any glycogen. No appreciable amounts of glycogen were discovered in the suspension solutions at the end of an eschatin experiment (*Exp.* 200, table 1). As a further control, instead of adding 1.5 cc. eschatin, 1.5 cc. 0.9% NaCl solution, or the same containing

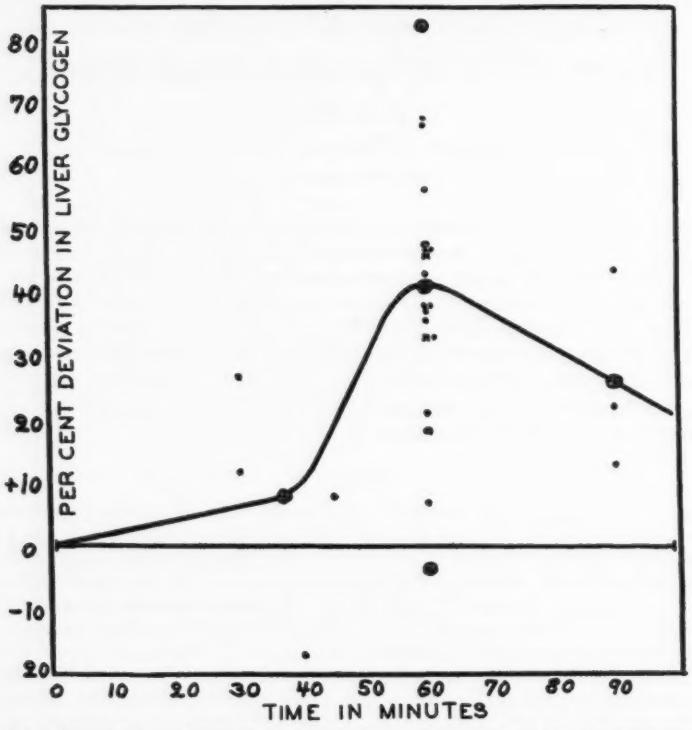

Fig. 1. INFLUENCE AT VARYING TIMES AND CONCENTRATIONS OF ESCHATIN ADDED IN VITRO UPON GLYCOGENOLYSIS OF SURVIVING RAT LIVER SLICES IN PERCENTAGE DEVIATION FROM HORMONE-FREE STANDARD GLYCOGENOLYSIS. The latter is represented by the zero x-line. Concentrations of eschatin per 5 cc. suspension solution: circles = 4 R.U.; dots = 6 R.U.; crosses = 8 R.U.; circled crosses = group averages connected by smoothed curve; circled dots = female rats (see table 1).

1:1,000,000 adrenalin were added to the suspension solution without affecting the standard rate of hepatic glycogenolysis (*Exp.* 219).

'Zero' glycogen figures and standard glycogenolysis. Liver glycogen figures as observed 20 to 28 minutes after death varied between 3.9 and 6.2 gm. %. Average deviations from mean in the duplicate determinations of both the zero samples and the suspended slices, were the same as given in the two earlier publications (1, 2). The time curve of the hormone-free standard glycogenolysis of the suspended rat liver slices, starting 20 to 28 minutes after death, was a little shallower in the present experiments than in figure 1 of the second communication (30 to 40 minutes, −20%; 60 minutes, −38%; 90 minutes, −42%, average).

Glycogenolysis with eschatin added in vitro. The data collected in 29 determinations

are presented in their actual order in table 1. In figure 1, the percentage deviations of eschatin-glycogenolysis from hormone-free standard glycogenolysis are arranged according to time. With only two exceptions, all figures observed in the eschatin series show an inhibition of hepatic glycogenolysis, indicated by plus deviations from the standard figure of each experiment. The maximum inhibitions by eschatin were seen at 60 minutes and 6 R.U. per 5 cc. of suspension solution; 2 and 4 R.U. were ineffective

TABLE 1. INFLUENCE OF VARYING CONCENTRATIONS OF ESCHATIN ADDED IN VITRO ON THE GLYCOGENOLYSIS OF SURVIVING RAT LIVER SLICES SUSPENDED FOR VARYING TIMES IN A BUFFERED SALT SOLUTION (PH 7.4, T. 37° C.)

No. of exper.	Concentr. of eschatin	Time	Liver glycogen content						
			A. No eschatin			B. Eschatin		Difference in glycogenolysis between A and B	
			At start	At end	Diff.	At end	Diff.		
	R.U. per 5 cc.	min.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	mg./gm.	%
184	2	60	47.89	32.88	−15.01	33.28	−14.61	+ 0.40	+ 3.0
	4	60				35.65	−12.24	+ 2.77	+18.5
185	4	60	62.13	37.73	−24.40	39.37	−22.37	+ 1.64	+ 6.7
186	6	60	55.39	34.71	−20.68	42.08	−13.31	+ 7.37	+35.6
	8	60				41.61	−13.78	+ 6.90	+33.3
187	6	30	49.15	40.89	− 8.26	41.86	− 7.29	+ 0.97	+11.7
	6	60		32.94	−16.21	38.96	−10.19	+ 6.02	+37.2
188	6	60	47.48	32.05	−15.43	39.26	− 8.22	+ 7.21	+46.8
	6	90		29.21	−18.27	33.30	−14.18	+ 4.09	+22.3
200	6	60	41.14	22.76	−18.83	29.94	−11.20	+ 7.18	+38.2
	6	60				28.05[1]	−13.09	+ 5.74	+30.5
201	6	45	43.31	33.53	− 9.78	34.31	− 9.00	+ 0.78	+ 8.0
	6	60		29.90	−13.41	34.99	− 8.32	+ 5.09	+38.0
202[2]	6	60	45.87	30.76	−15.11	43.21	− 2.66	+12.45	+82.5
203[3]	6	60	56.32	38.78	−17.54	38.15		− 0.63	− 3.6
204	4	60	46.80	28.30	−18.50	37.08[3]	− 9.72	+ 8.78	+47.5
	6	60				36.27	−10.53	+ 7.97	+43.1
	8	60				36.80[3]	−10.00	+ 8.50	+46.0
205	6	60	38.76	23.66	−15.10	33.74	− 5.02	+10.08	+66.4
	6	90		22.62	−16.14	29.62	− 9.14	+ 7.00	+43.5
206	0[4]	60	44.67	25.68	−18.99	35.11[3]	− 9.56	+ 9.43	+49.6
	6[4]	60				34.56	−10.11	+ 8.88	+47.1
	6	60				38.53[3]	− 6.14	+12.85	+67.6
207	6	40	44.93	36.00	− 8.93	34.13	−10.80	− 1.87	−17.3
	6	60		28.10	−16.83	31.66	−13.27	+ 3.56	+21.2
208	6	30	41.55	31.83	− 9.72	34.34	− 7.21	+ 2.51	+26.8
	6	60		25.52	−16.03	30.92	−10.63	+ 5.40	+33.8
	6	90		22.47	−19.08	24.97	−16.58	+ 2.50	+13.2
209	6	60	46.75	23.90	−22.85	36.73	−10.02	+12.83	+56.0
210	6	60	48.72	27.06	−21.66	37.34	−11.38	+10.28	+47.3

[1] Tissue analyzed with suspension solution.
[2] Female rat.
[3] No duplicate determinations.
[4] Plus 4 U. of insulin per cc.

or inconsistently effective whereas 8 R.U. did not do more than 6 R.U. The average inhibition of rat liver glycogenolysis at all hormone concentrations was +8% at 30 to 45 minutes, i.e., on the upper limit of experimental error; +41.2% at 60 minutes; and +26.3% at 90 minutes (see curve in fig. 1). Among the group examined at 60 minutes and 6 R.U. concentration, the two extreme figures of −3.6 % and +82.5% inhibition were found in the only 2 female rats in the experimental series, whereas the range among the males was between +21.2 and +67.6%. The

sexual cycle of the female rats had not been determined. According to the literature, the corpus luteum hormone (progesterone) exerts an influence synergistic to that of the adrenal cortical hormone (5).

The inhibitory effect upon rat liver glycogenolysis of eschatin was found to be greater than that of insulin (average at optimum time and concentration +34%, 2). The combined in vitro effect of both hormones was, however, not greater than that of insulin alone and smaller than that of eschatin alone (*Exp.* 206, table 1).

DISCUSSION

Most investigators agree that the adrenal cortical hormone is a fundamental factor in controlling carbohydrate metabolism of mammals from opossum to monkey (6–9), with the possible exception of the dog (10). In the absence of the adrenals, more than 50% of the total carbohydrate material in the body may be lost (8, 11). Extreme hypoglycemia with or without fatal convulsions is typical of adrenalectomized animals (12, 13, 14 and others). At the same time, liver glycogen drops to exhaustion values and skeletal as well as cardiac muscle glycogen is markedly reduced. The animal loses the ability to mobilize glycogen and to synthesize it from glucose, lactic acid and non-carbohydrate sources (7, 14, 15, 16). As soon as cortical hormone is supplied to adrenalectomized animals, complete reversal from 'glycogenetic failure' (8) to normal carbohydrate metabolism is seen in a short time. Furthermore, in normal fasting rats, rabbits and cats, cortical hormone produces a definite rise in blood sugar and liver and muscle (?) glycogen (16, 17). In both adrenalectomized and normal fasting animals, after the injection of cortical hormone, a decrease in peripheral carbohydrate oxidation, i.e., a drop in R.Q., and an excretion of extra-nitrogen in the urine is observed (14, 16).

On the basis of these facts, it has been suggested that cortical hormone acts on carbohydrate metabolism in three different ways: (*a*) by decreasing the peripheral utilization of glucose within the tissues; (*b*) by increasing the formation of glucose from lactic acid and non-carbohydrate sources; (*c*) by synthesizing and fixing glucose as liver and muscle glycogen ('glycostatic' or 'glycopexic' function, Britton 18).

The inhibition of in vitro glycogenolysis of rat liver slices by cortical hormone is in good agreement with the hormone's effect on in vivo carbohydrate metabolism. Of the three mechanisms just referred to the first and second are improbable of materialization in surviving liver tissue because of the predominance under post-mortem conditions of the catabolic processes such as oxidation, glycogenolysis, proteolysis. This leaves us with the third possibility which, in actual fact, involves an inhibition of the glycogenolytic enzyme of the tissue cells. According to Willstätter, Przylecki and other enzymologists (19, 20), inhibition of hepatic glycogenolysis by hormones is due to their blockading the intracellular activators of the glycogenolytic enzyme (peptids, amino-acids, etc.) and to consequent preponderance of the intracellular inhibitors of the enzyme (proteins, amines, etc.). It is also conceivable that the hormones may further readsorption of the enzyme to intracellular surface structures.

This action of cortical hormone is similar and, in terms of degree, superior to the action of insulin tested under identical conditions. Our in vitro experiments confirm Britton's in vivo results marking "the adrenal cortex as a highly effective glycopexic mechanism apparently outranking insulin" (18).

No in vitro experiments testing the effect of cortical hormone are recorded in the literature. Viale (21) examined post-mortem glycogenolysis of the muscles of adrenalectomized dogs (37° C., 2 hours) and often found an inhibition of that process. Britton and Silvette (17) have announced 'tests on excised tissues' for assaying cortical

hormone as being in progress and offering considerable promise, but no results have been so far presented. Perhaps the technic described in this paper could be used for the purpose of assaying the hormone (male rats; 6 R.U. per 5 cc. suspension solution; 60 minutes experiment, starting 25 minutes after death; expected effect 5 cc. 30 to 50% inhibition of hepatic glycogenolysis).

The significance of the results presented in this paper for the pathogenesis of Von Gierke's glycogen disease will be discussed later.

SUMMARY

With an in vitro method described in earlier publications, rat liver glycogenolysis, as it proceeds in surviving tissue slices suspended in a buffered salt solution, was found to be inhibited by large doses of cortical hormone (eschatin) added in vitro. The degree of inhibition averaged about +41% at a 60-minute interval and a concentration of 6 R.U. per 5 cc. (fluctuation for male rats +21 to +67%).

In female rats the results seem to be influenced by the hormonal tides of the sexual cycle (two experiments with −3 and +82% inhibition).

The inhibitory effect on hepatic glycogenolysis of cortical hormone is greater than that of insulin added in vitro and cannot be increased by combining the two hormones.

If in vitro experiments bear any relationship to in vivo physiology it is to be concluded that one essential function of the cortical hormone consists in an inhibition of the glycogenolytic enzyme of the liver cells ('glycopexic' function of Britton's). The observations made in the living experimental animal are in good agreement with this conclusion.

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THE METABOLISM OF NITROGEN, SULFUR AND GLUTATHIONE IN THYMECTOMIZED ALBINO RATS

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THOUGH THE thymus gland has attracted interest for more than three hundred years (1) and was actively investigated even before the middle of the last century, its accurate place in the endocrine pattern of the organism is still to be determined.

In a previous study on the influence of thymectomy on the mineral metabolism of rats (2) we showed that the removal of the thymus does not interfere with calcium, phosphorus and magnesium metabolism. The only change which was found at that time was an increase in urinary nitrogen excretion. Since this increase was accounted for by urea nitrogen, while ammonia nitrogen, uric acid, creatinine and creatine remained unchanged, it was concluded that the disturbance in nitrogen metabolism was confined to protein catabolism. In order to obtain additional information on the influence of the thymus on the utilization of protein, sulfur metabolism was studied in detail, including the sulfur bearing tripeptide glutathione, on which recent work has focused attention in connection with the thymus gland (3).

EXPERIMENTAL

Thirty-two male and female albino rats (Wistar strain) were used in the experiment. The rats were kept in groups of 3 in metabolism cages as described in a previous publication (4). Four rats of each sex were kept as paired feeding controls. Urine and feces were collected twice a week. The urine was analyzed for total nitrogen (Kjeldahl), total sulfur (5) and total and inorganic sulfates (Folin). Feces and food were analyzed for total nitrogen (Kjeldahl) and total sulfur (6). Parallel groups of litter mates of the experimental animals were used for glutathione determinations (7). The rats were studied for a control period of 2 weeks before thymectomy was performed at the age of 40 days, and for 18 weeks thereafter. The figures mentioned in the discussion are averages of the results obtained on all the animals, while a typical protocol is presented in the tables.

DISCUSSION

Though it has not yet been possible to ascertain the specific function of the thymus, there is little doubt that it is related to that of several other glands of internal secretion. Marine (8) states that it is certain that there is an important indirect relation between thyroid and thymus through the sex glands and suprarenals, and both the thyroid and the thymus are usually affected in conditions involving the suprarenals or sex glands, as for example Addison's disease, status lymphaticus, castration and Graves' disease. Comsia (9) found that in young male guinea pigs thymectomy retards growth and interferes generally with the well being of the animals. Gershon-Cohen and his coworkers (10) involuted the thymus in young

rats by x-rays, and report that this treatment results in an arrest of spermatogenesis and the development of a typical castrate pituitary picture up to the age of 100 days. After this period reparative processes set in so that in 50 to 100 days more, spermatogenesis is restored and the pituitary has returned to normal. These changes, however, are restricted to males. Similar destruction of the thymus in females produces no functional or anatomic change in the ovary or pituitary.

We did not observe any sex-bound difference in the response to thymectomy as far as protein metabolism is concerned. Therefore, data on males only are presented in the tables.

Urinary nitrogen excretion increased progressively from 13% of the intake during the control period to 39% 10 weeks after thymectomy, and was still 37% 18

TABLE 1. NITROGEN METABOLISM IN THYMECTOMIZED RATS DAILY AVERAGE BY WEEKS

Date	Total nitrogen excretion			Intake	Retention
	Urine	% of Intake	Feces		
	mg.		mg.	mg.	mg.
Control period					
Oct. 25–31, 1938	42	10	93	406	271
Nov. 1–7	63	14	105	439	271
Thymectomized on Nov. 8th					
Nov. 11–17	118	23	121	518	279
18–24	95	18	126	538	317
25–Dec. 1	104	20	118	541	319
2– 8	122	23	122	528	284
9–15	129	25	123	508	256
16–22	124	20	103	521	294
23–29	145	30	91	464	228
1939					
30–Jan. 5	140	35	83	427	204
6–12	143	37		391	179
13–19	165	40		418	162
20–26	128	36	84	418	206
27–Feb. 2	130	31	76	423	217
3– 9	148	36	82	408	178
10–16	161	41	80	396	155
17–23	133	35	67	381	181
24–Mar. 2	171	35	91	483	221
3– 9	173	35	80	487	234
10–16	184	40	81	467	202

weeks after operation when the experiment was terminated. In the paired feeding controls urinary nitrogen excretion increased from 15% of the intake during the control period to 24% only during the course of the experiment. Fecal nitrogen excretion, expressed in percentage of intake, remained unchanged in both groups. This increase in urinary nitrogen excretion is of exactly the same order of magnitude as that which we observed in rats castrated before puberty (11).

Total urinary sulfur excretion increased from 10% of the intake during the control period to 32% 10 weeks after thymectomy, and remained at an average of 30% for the duration of the experiment. In the paired feeding controls, total urinary sulfur excretion increased from 11% to 18% only during this period. While the disturbance in nitrogen metabolism is confined to protein catabolism, endogenous as well as exogenous sulfur metabolism is affected by the removal of the thymus gland, as indicated by a steady rise in the excretion of neutral sulfur, which increases from 25% of total urinary sulfur before thymectomy to an average of 40%, while in the controls no such change takes place. The excretion of inorganic and

TABLE 2. SULFUR METABOLISM IN THYMECTOMIZED RATS DAILY AVERAGE BY WEEKS

Date	Total sulfur excretion			Intake	Retention	Neutral S	% of Urinary S excretion	Sulfates	
	Urine	% of Intake	Feces					Inorgan.	Ethereal
	mg.		mg.	mg.	mg.	mg.		mg.	mg.
Control period 1938									
Oct. 25–31	2.3	9	8.8	26.5	15.4	0.5	22	1.5	0.3
Nov. 1– 7	3.4	12	10.3	28.5	14.8	0.9	27	2.2	0.3
Thymectomized on Nov. 8th									
Nov. 11–17	5.1	15	12.5	33.6	16.0	0.9	18	3.6	0.6
18–24	4.8	14	13.7	34.9	16.4	0.8	17	3.3	0.7
25–Dec. 1	5.1	15	12.9	35.1	17.1	1.6	31	2.9	0.6
2– 8	5.3	15	13.4	34.2	15.5	1.9	36	2.8	0.6
9–15	6.3	19	11.1	33.8	16.4	2.3	37	3.5	0.5
16–22	6.2	19	12.3	33.0	14.5	2.7	44	3.2	0.3
23–29	7.7	27	10.2	28.3	10.4	3.3	43	3.9	0.5
1939									
30–Jan. 5	6.9	26	9.6	26.1	9.5	2.8	41	3.5	0.6
6–12	6.7	28	6.9	23.9	10.3	2.7	40	3.4	0.6
13–19	8.1	32	9.5	25.6	8.0	3.4	42	4.1	0.6
20–26	6.4	27	9.8	25.6	9.4	2.6	41	3.2	0.6
27–Feb. 2	6.5	24	9.6	27.0	10.9	2.9	45	3.1	0.5
3– 9	7.3	29	10.3	25.5	7.9	2.8	40	3.7	0.8
10–16	7.8	31	10.0	24.8	7.0	3.0	38	4.1	0.7
17–23	6.1	26	7.7	23.9	10.1	2.4	40	3.3	0.4
24–Mar. 2	8.9	29	10.2	30.3	11.2	3.5	39	5.0	0.4
3– 9	9.4	31	9.3	30.5	11.8	3.9	41	5.3	0.2
10–16	9.9	34	9.4	29.2	9.9	4.2	42	5.0	0.7

ethereal sulfates, expressed in percentage of total urinary sulfur, as well as fecal sulfur excretion, expressed in percentage of intake, remains unchanged.

The striking similarity in the changes in nitrogen as well as in sulfur metabolism presented by thymectomized and castrated animals (11) seems to support the assumption of a relationship between the thymus and the gonads. A comparison of the nitrogen and sulfur metabolism of thymectomized rats with that of animals deprived of either pituitary (12, 13) or suprarenals (14) is more difficult to evaluate on account of the diuresis resulting from the removal of those glands, but the changes proceed in the same direction.

The work of Rowntree and his coworkers (15) indicated a possible rôle of glutathione in the function of the thymus. Stekol (16) suggested that the retardation of growth in the offspring of thymectomized rats may perhaps be due partly to a lack of glutathione, since it has been found that glutathione is utilized as such by the rat as well as that it exerts a sparing action on that dietary cystine and methionine

TABLE 3. GLUTATHIONE IN BLOOD BEFORE AND AFTER THYMECTOMY

Date	Total glutathione	Reduced glutathione	Oxidized glutathione
	mg./100 cc.	mg./100 cc.	mg./100 cc.
Before thymectomy	42	32	10
After thymectomy			
2 weeks	46	30	16
4 weeks	47	25	22
6 weeks	43	25	18
8 weeks	43	25	18
18 weeks	42	31	11

which are used for the synthesis of new protein in the tissues and for other anabolic purposes.

Our experiments show that while total glutathione in the blood tends to rise after thymectomy, the increase is limited to 12% of the normal amount and persists only for about a month after operation. Then it begins to drop and has reached almost normal values within 2 to 3 weeks. But while the change in the total glutathione content is slight, there is a pronounced disturbance in the ratio between reduced and oxidized glutathione. Reduced glutathione shows a drop of 22% of the normal value. This drop develops slowly and is apparent only 4 weeks after the removal of the thymus gland but is still present 8 weeks after operation. The most striking disturbance, however, is encountered in the oxidized part of glutathione. Two weeks after thymectomy it shows a rise of 60% from the normal. The peak is reached at 120% of the original value 4 weeks after operation. Then a slow decrease sets in, but a month later the rise still amounts to 80% of the normal. During the third month after thymectomy adjustment begins to take place and 18 weeks after operation total glutathione as well as the ratio between reduced and oxidized glutathione have returned to normal. Our findings on the behavior of glutathione in thymectomized animals agree with the assumption of a relationship of the thymus to other glands of internal secretion. The changes in oxidized glutathione coincide as far as the time factor is concerned with the disturbance in gonadal function observed by Gershon-Cohen (10), but were found in both sexes. Rivoire and Bermond (17) draw attention to the fact that oxidized glutathione disappears, often completely, from the blood of patients suffering with Graves' disease, while oxidized glutathione rises considerably in our thymectomized animals. Endogenous sulfur metabolism in thymectomized rats follows the same pattern as in suprarenalectomized animals and our observation of a drop in reduced and pronounced increase in oxidized glutathione in thymectomized rats agrees with the findings of Binet and Waller (18) that in Addison's disease there is a drop in reduced glutathione while oxidized glutathione increases.

SUMMARY

The influence of thymectomy on the metabolism of nitrogen and sulfur including the sulfur bearing tripeptide glutathione was studied in male and female albino rats. Urinary nitrogen excretion increases progressively, fecal excretion remains unchanged. Exogenous as well as endogenous sulfur metabolism is disturbed. Total urinary sulfur as well as neutral sulfur excretion increases. Fecal sulfur, inorganic and ethereal sulfate excretion remains unchanged. Total glutathione in the blood of thymectomized rats rises slightly, but begins to return to normal 6 weeks after operation. A drop in reduced glutathione develops more slowly, but persists longer. The main disturbance is encountered in oxidized glutathione, which rises considerably after operation, and 2 months later still shows high values. But eventually adjustment takes place and 18 weeks after thymectomy all glutathione values have returned to normal. The interrelationship between the thymus and other glands of internal secretion has been discussed.

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THE RELATIONSHIP BETWEEN THE THYMUS AND THE SEXUAL ORGANS

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In 1898 Calzolari (1) observed that the thymus of rabbits castrated before sexual maturity was larger than that of the controls. This was confirmed by numerous workers and interpreted as a delay in the physiological involution. Moreover, the inverse effect, i.e. atrophy of the thymus, was found to be produced by the injection of sex hormones or of gonadotropic substances. These facts, together with the hypertrophy caused by adult castration, would indicate that the involution of the thymus is determined by the activity of the sexual organs. An important point, the determination of the exact moment at which involution is initiated and also the prepuberal changes of the thymus in animals castrated before sexual maturity, has generally been disregarded.

Our plan has been to determine the growth curve of the thymus in normal animals or castrated, either before, during or after sexual maturity. As individual variations in thymus weight are large, a great number of animals was used, and special attention was paid to those factors which can determine an accidental involution. The influence of adult castration, sex hormones, thyroidectomy and adrenalectomy was also studied.

METHODS

Albino rats from the stock colony of the Physiological Laboratory of Buenos Aires were used. These animals show fairly constant morphological characters. From 10 to 20 rats were lodged in Portland cement cages (50×50×50 cm.), the floor being covered with a layer of sawdust. Temperature was not allowed to fall below 20° C. The animals received a liberal daily diet composed as follows: ground maize 1 kg.; wheat 1 kg.; white bread 5 kg.; dried meat 0.5 kg.; milk 8 liters.

When approaching the final stage of gestation, the females were transferred to individual cages in order to ascertain the exact birth date of the sucklings. These were kept with their mother up to the 30th day. Castration was performed under ether anesthesia, in males by incision of the pouch, in females by via dorsalis. Controls from the same litter were only anesthetized. At a variable time after the operation, the animals were killed with coal gas, the thymus was immediately removed, carefully freed from connective tissue, weighed on a torsion balance, and finally dropped into a 10% formol solution.

The influence of castration on the thymus. Castration can be performed either before or after sex maturity. The weight of the thymus has been found to be greater in animals after prepuberal castration (1–5). Prepuberal examination of these animals revealed no changes according to Gellin (6) and Paton (7). By castration after sex maturity the thymus has also been found to be larger than in the controls (6, 8, 9, 10). According to other experiments, the greater weight of the thymus would be due to a delay caused by castration in the physiological involution of the organ (1, 6, 8, 9, 11–14). This interpretation appears to be confirmed by histological studies (1, 6, 8).

According to these results, it was admitted that prepuberal castration determines a fairly

evident delay in the beginning of thymic involution and that sex maturity constitutes the causal agent of atrophy. Hammar (15), in his last work (1936), states that the physiological involution of the thymus, which is accompanied by the reduction of lymphatic tissue of the organism, was especially due to the depressive action of substances poured into the blood by the gonads. As they are lacking during the prepuberal period, gonadectomy exerts no influence upon the thymus before reaching that state.

EXPERIMENTAL RESULTS

Males. Ninety-two male rats were castrated at 30 to 35 days of age. The 92 controls of the same litter were only anesthetized. Each pair of animals was left together during the experimental period. At the age of 45, 60, 70, 80, 100, 120, 140, 160, 180, 200 and 250 days, several pairs of rats were killed.

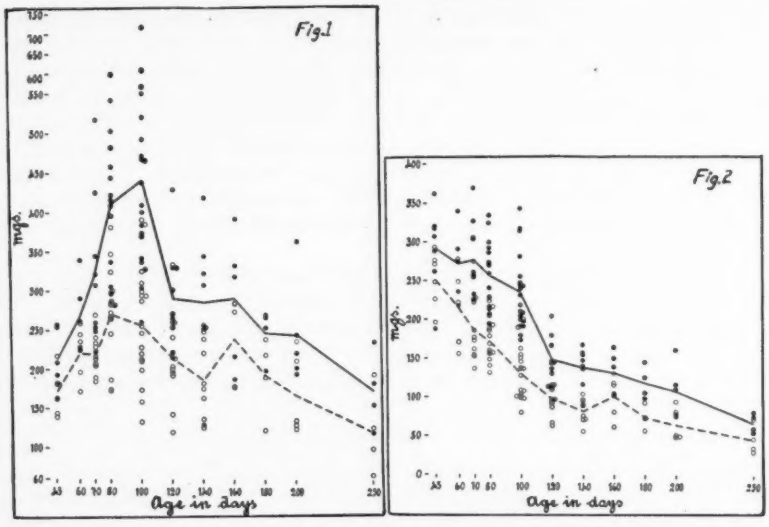


FIG. 1. ABSOLUTE THYMUS WEIGHT OF MALE RATS, CASTRATED BEFORE PUBERTY (30 days of age). FIG. 2. THYMUS WEIGHT OF MALE RATS, CASTRATED BEFORE PUBERTY (related to 100 gm. of body weight). Dots indicate castrate animals; circles, normal rats. Solid line indicates arithmetical average of castrates; broken line, average of normals.

The body weight of the castrated animals was always equal to or lower than that of the controls. The means of absolute weights of the thymus are plotted in fig. 1. The thymus weight of the castrated animals was always higher than that of the controls, the difference reaching its highest value between 80 and 100 days. In the castrated animals the involution commences at 100 days, suffering a slight delay as compared with the controls in which it begins at 80 days.

Another fact worth mentioning is that the rate of weight loss is greater in the castrated animals. Figure 2 shows the differences between the absolute thymus weights of castrated and control animals. From this it is apparent that the difference is really significant. If instead of comparing the absolute weights we compare the weight of the thymus referred to body weight, we still find important differences (fig. 2).

Two additional experimental series were carried out, using rats from another stock. Results agreed with those already described, proving that the changes were not confined to that particular breed only.

Females. Sixty-six rats were castrated at 30 days of age. The corresponding con-

trols, 71, were from the same litter. The experiment was carried out in exactly the same way as for the male animals. The body weight of the castrated animals was nearly always above that of the controls. Figure 3 shows the average weight curve of the thymus. The weight is almost equal up to 45 days. From then on the thymus weight of the castrated animals becomes higher, reaching the maximum difference at 70 days. After this peak there is an abrupt decrease between 70 and 100 days. The curves are almost parallel, but that corresponding to the castrated animals is higher and its descending portion begins later (10 days). If the relative weights of thymus are compared, those of the castrated are also found to be higher than those of the controls.

If we compare the thymus weight of the castrated males with that of the females, the only difference observed is that the maximum is reached earlier in the latter (70

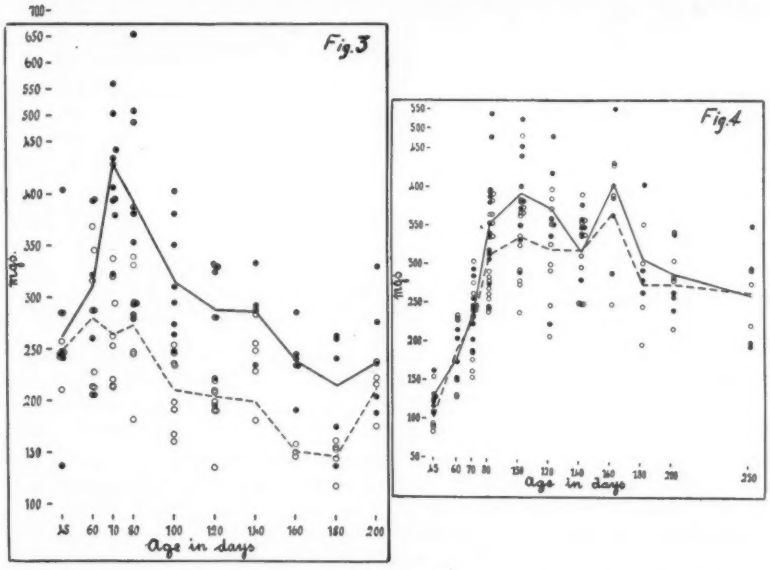


FIG. 3. ABSOLUTE THYMUS WEIGHT OF FEMALE RATS, CASTRATED BEFORE PUBERTY (30 days of age).
Fig. 4. ABSOLUTE WEIGHT OF GROUPS OF LYMPHATIC GANGLIONS (submaxillary, cervical and axillary) OF MALE RATS, CASTRATED BEFORE PUBERTY. Solid dots indicate castrate rats; solid line, average of castrates; circles indicate normals; broken line, average of controls.

days) than in the former (100 days). Referred to body weight, the weight of the thymus of the castrated females is higher at 45 to 80 days, but later on it is nearly equal to that of the males.

In a certain number of the prepuberally castrated rats the quantitative relation of cortex to medullae of the thymus was determined following Hammar's technic. By comparing the values thus obtained from 186 cuts belonging to the thymus of 16 prepuberally castrated males with those of 14 controls of 80, 100 and 120 days, it was found that there was a greater proportion of cortex in the castrated animals of 80 days. At 100 days the difference was slight and then disappeared (table 1).

Histological examination showed that in the normal, as well as in the castrated animals, the involutive process begins after the 80th day, varying in its intensity at the 100th day, according to the animals; but in both it becomes fairly evident and uniform at 120 days of age.[1]

[1] For the histological study, I am indebted to Dr. Jorge Porto.

TABLE 1

Age	Castrated			Normal		
	Thymus weight	Number of cuts	% Cortex	Thymus weight	Number of Cuts	% Cortex
days	*mg.*			*mg.*		
80	459	13	80,11	301	11	75,57
	445	11	78,90	287	8	75,09
	504	11	83,18	286	8	76,97
	543	3	77,67	—	—	—
100	552	5	73,27	288	5	72,24
	307	3	81,57	247	6	79,06
	261	3	76,22	311	5	80,28
	402	9	78,37	200	8	75,28
	385	8	78,38	—	—	—
	375	3	78,77	—	—	—
	411	6	77,60	—	—	—
120	431	10	74,17	261	7	78,30
	260	7	70,02	143	3	74,34
	216	5	79,37	205	2	79,43
	—	—	—	120	4	65,18
200	223	3	76,01	130	5	76,97
	246	9	75,03	213	5	73,96

Effect of Prepuberal Castration Upon Different Groups of Lymph Nodes in Males and Females

As some authors consider the thymus as belonging to the lymphatic system, it seemed desirable to establish the relationship between lymphatic ganglions and thymus. The most differentiated lymphatic ganglions (submaxillary, cervical and axillary) of both prepuberal castrated males and females and of their corresponding controls were weighed. The absolute weights are plotted in figure 4.

In normal rats the growth of the lymphatic ganglions reaches its maximum at 80 days and involution commences at 160 days. In castrated rats of both sexes a slight hypertrophy of the ganglions is observed.

Effect of Castration in Adult Age

Following the above method, 40 males of 120 days of age were castrated. Thirty-five normal rats of the same litter and sex were used as controls.

Figure 5 shows that the thymus weight of the gonadectomized animals is already increased after 30 days. The increase still continues at 45 and 60 days after operation, that is, at 150 and 180 days of age respectively. The increase is equally apparent, when thymus is referred to body weight (mg. per 100 gm.). As the weights, both absolute and relative, in the 42 female rats (of which 23 were castrated) of the same age as the male ones were similar to those obtained in the males, sex differences can therefore be discarded.

The histological examination did not reveal any appreciable qualitative differences, and only an increase in the quantity of thymus parenchyma was found.

Effect of Sex Hormones on Thymus

Fiore and Franchetti (16), Trentini (17) and Hammar (18) injected blood serum from mature into prepuberal animals of the same species and found that this leads to an atrophy of the thymus. These results, though not affording direct evidence, may

be attributed to the action of sex hormones. Ovarian grafts (19) have been shown to produce the same effect. In many experiments it has been found that the causes which favor intense development of sexual organs, lead to an atrophy of the thymus. This is the case with injections of placental and urinary extracts from pregnant women (20, 21, 22) as well as with anterior pituitary extracts (23).

The fact that Evans and Simpson and Klein found that the atrophic action of gonadotropic hormone or pregnancy urine on the thymus does not manifest itself in castrated animals, constitutes a proof of the action of the gonads. According to Watrin and Florentin (24) the effect of the anterior lobe of hypophysis is due to thyrotropic hormone.

The question arises as to which portion of the gonads, interstitial or germinative, is responsible for the atrophy of the thymus. According to Evans and Simpson (21), the degeneration of the germinative portion of the testicle due to experimental cryptorchidism or vitamin E deficiency does not prevent the effect of the gonadotropic

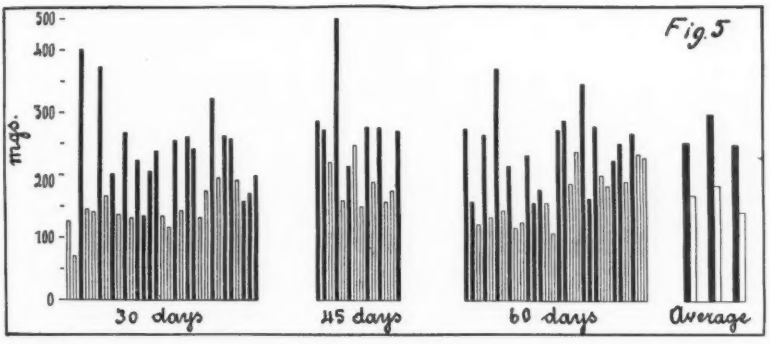


Fig. 5. Absolute thymus weight at intervals after operation of male rats castrated at 120 days age of (solid line, castrates; rectangles, normals).

extracts; therefore the interstitial portion with its Leydig's cells would be responsible for thymus atrophy. But due to the small number of animals employed, this cannot be accepted as an established fact without further confirmation.

Klein (22) agrees with Evans and Simpson with respect to this point of view, based upon the fact that the injection of gonadotropic hormones in his animals gave rise to an excitation of Leydig's cells only, without producing any appreciable changes in the germinative tissue of the testicle.

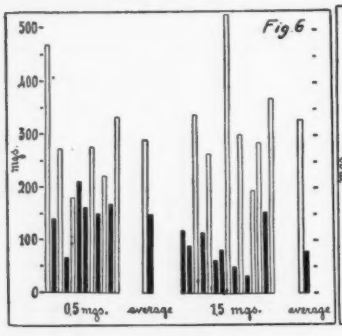
On the other hand, the injection of pure sex hormones, androsterone, testosterone, etc. (25) has an evident atrophic effect upon the thymus of normal and castrated rats, either prepuberal or mature, although its relationship with dose and type of involution caused by the administration of large quantities of hormones, is not sufficiently cleared up.

Histologically, the aspect of the thymus observed by Da Re (19) in female rabbits with ovarian graft, by Carrière, Morel and Gineste (23) in castrated and normal rats subsequent to injection of folliculin, by Ronsisvalle (20), Castagna (26) and Klein after injection of pregnant human urine or placental extracts in rabbits and rats, shows uniformly the disappearance of the thymic lymphocytes and intense atrophy of the cortical area, and therefore of the thymus lobe, which is substituted by fatty tissue. There occurs in addition a certain degree of proliferation of connective tissue. Korenchevsky and associates (25) did not find any histological changes in the thymus after

the injection of estrone in 8 normal albino rats. Similar results were obtained by Carrière, Morel and Gineste (23), when studying the effect of testosterone propionate on rats.

Male Sex Hormones

Castrated animals. Seven male rats and 6 females were castrated at the age of 45 days and from the 80th day onward they received daily subcutaneous injections of testosterone propionate (perandren, Ciba, 1.5 mg.) for 11 days. The controls, 8 males and 7 females, also castrated and belonging to the same litter, received injections of the solvent only (0.33 cc. of sesame oil per day). In another experimental series, 7 males, a smaller dose of testosterone was used (0.5 mg.). The animals were killed 4 days after the last injection. The thymus and the seminal vesicles were weighed. Figure 6 shows the decrease in absolute weight of the thymus, produced by



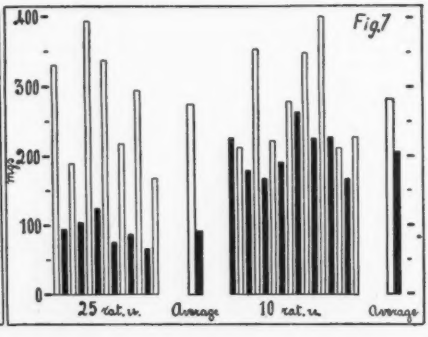


FIG. 6. EFFECT OF TESTOSTERONE PROPIONATE UPON ABSOLUTE THYMUS WEIGHT OF MALE RATS, CASTRATED BEFORE PUBERTY (solid line, testosterone injected; rectangles, oil controls). Fig. 7. EFFECT OF ESTRONE UPON ABSOLUTE THYMUS WEIGHT OF MALE RATS CASTRATED BEFORE PUBERTY (solid line, estrone injected rats; rectangles, oil injected controls).

testosterone in males. The effect is somewhat less marked in the case of the lower dose.

Normal animals. For a period of 11 consecutive days, 7 normal males of from 75 to 79 days of age, and 7 female rats of from 100 to 115 days of age were given daily injections of 1.5 mg. of testosterone propionate. The controls, 6 males and 6 females from the same litter, received injections of the solvent only. When killed, 7 days after the last injection, a thymus atrophy, similar to that of castrated animals, was found.

Female Sex Hormones

Castrated animals. For a period of 11 days from the 80th day on, 7 males castrated between 41 and 43 days of age were given daily injections of 25 R.U. of estrone diluted in 0.25 cc. of oil-glycolethylene solution. Eight other males castrated at 46 days of age received, from the 94th day on, for 7 days, 10 R.U. of estradiol dissolved in 0.05 cc. of sesame oil. The controls, 14 males from the same litters, received injections of the solvent only. Necropsy was performed 4 days after the last injection.

For 12 consecutive days from the 85th day on, 6 females received daily injections of 25 R.U. of estrone. The latter animals had been castrated at between 41 and 43 days of age.

Eight additional females, castrated at 47 days of age, received daily injections of 10 R.U. of estradiol, dissolved in 0.05 cc. of sesame oil, from the 80th day on for 7 days. As controls for both groups 15 castrated females from the same litters were

used. These were given injections of the solvent only. Necropsy was performed the day after the last injection.

Injection of female hormone originated decrease of the thymus size, which is more definite when larger doses are given (fig. 7). Similar results were obtained when referring thymus weight to 100 gm. of body weight.

Normal animals. For these experiments we used 4 male albino rats of 85 days of age and 6 of from 104 to 113 days of age; for 11 consecutive days, these animals received daily injections of 20 R.U. of estradiol. The controls from the same litters, 12 males, received injections of the solvent only. Autopsy was performed 4 days after the last injection. These animals did not show any appreciable decrease in thymus weight.

Seven female rats of from 89 to 99 days of age were given daily injections of 20 R.U. of estradiol for 11 days. Seven females from the same litters, receiving the solvent only, served as controls. Post-mortem examination was made 2 days after the last injection.

As to both absolute and relative values, there was an average decrease of thymus weight in the animals which had received female hormone injections.

Histological examination of the thymus of animals which had received male or female hormone injections, revealed an intense atrophy of the thymus lobules, especially in the cortex. This was due to the intense destruction of the thymic lymphocytes, the rest of which formed irregularly disseminated masses. There was an increase of connective tissue, especially around the blood vessels, the walls of which were thickened. The intra- and interlobular fat was more abundant than in the corresponding controls.

Effect of Thyroidectomy and Adrenalectomy Upon the Thymus in Prepuberally Castrated Albino Rats

Thyroidectomy in castrated animals. For these experimental series rats of known age, castrated between 30 and 40 days of age, were used.

Nine male animals had been thyroidectomized between 50 and 60 days of age; necropsy took place 30, 43 and 67 days later. No signs of thyroid regeneration could be detected. In 12 out of 19 females, castrated between 30 and 40 days of age and thyroidectomized between 60 and 68 days of age, autopsy was performed at 28, 40 and 64 days after the last operation. No signs of thyroid regeneration could be detected. Controls from the same litters were only castrated.

The thymus of the castrated thyroidectomized rat is smaller in size than that of the castrated but not thyroidectomized animals. The same holds for those in which regeneration of thyroid took place. This holds for both absolute and relative values.

Histological examination revealed in the thymus of castrated and thyroidectomized rats a marked decrease in the size of the thymic lobules and an increase of the fatty interstitial connective tissue. It was not possible to appreciate any clear difference in the proportion of cortex to medulla between experimental and control animals.

Adrenalectomy in prepuberal castrated females. In 70 female albino rats, castrated at 30 days of age, simultaneous extirpation of both adrenals (via dorsalis) was performed under anesthesia with ether, when the animals reached 80 and 120 days of age. The animals recovered soon after the operation.

Symptoms of adrenal insufficiency appeared between 10 and 20 days following operation (loss of weight, limitation of motion, tremor); at this time mortality reached its highest degree; only 20% of the operated animals survived more than 30 days.

Nine of the 14 animals which survived belonged to the adrenalectomized at 80

days; they were killed at 120 days of age; the remainder (6 animals) belonged to those operated on at 120 and killed at 150 days of age. The castrated controls were in all cases from the same litter as the experimental animals, and were submitted to identical surgical traumatism.

Comparing the absolute individual values of the thymus, we find no appreciable differences between the castrated adrenalectomized rats and their castrated controls. In the adrenalectomized animals there exists a certain predominance of the average weight of thymus, which is more marked when relative values are compared.

In these experiments more importance should be given to the relative weight of thymus than to the absolute, because the body weight of the adrenalectomized rats was almost always less than that of the controls.

DISCUSSION

The growth curve of the normal thymus is shown in figures 1 and 2. The maximum is reached at 80 days of age in the males and at 60 in the females. The curve of the absolute thymus weights of the castrated animals is similar to that of the normal animals, but the growth period seems to persist 20 days longer than in normal males, and 10 days in the females. But that which characterizes the curves of the gonadectomized of both sexes is their height, notably superior to that of the normal animals during all ages between 45 and 250 days for the males, 45 and 200 days for the females. This difference between castrated and control animals reaches its highest degree at from 80 to 100 days in the males and at from 70 to 80 days in the females.

This outstanding increase when referred to 100 gm. of body weight of the thymus is not simply due to the greater development of the organism as it persists in both sexes. Individual variations of such magnitude and constancy can be discarded, as well as predisposition of certain breeds of rats, due to the fact that the controls were the brothers of the experimental animals from the same litter, and also because of the similar results arrived at in our experiments with rats from a different breed. Neither can it be attributed to an increase of fatty tissue, which is always more abundant in the castrated animals. Fatty acids were estimated (Bloor's method) in the hypertrophied thymus of 3 castrated prepuberal male rats, killed at 80 days of age. No appreciable difference between the total content of fatty acids in experimental and control animals was found.[2]

Histological examination showed that hypertrophy was due to the increase of the parenchyma itself. Compared with the controls, no gross changes in the fatty tissue of castrates could be seen. Though there existed a higher percentage of thymic cortex in gonadectomized males of 80 days of age, it was not large enough to deduce the definite conclusion that hypertrophy occurred at the expense of the lymphoid portion only, without the participation of the epithelial part of the parenchyma.

Thus we can conclude that the weight increase of the thymus of prepuberally castrated rats of both sexes is due to actual hypertrophy of the organ.

The physiological involution of the thymus was faster in the castrated animals than in the normal; the thymus of the castrated males lost 34% of its weight between 100 and 120 days, while that of the normal animals lost 18% in the same time. The thymus of the castrated females lost 26% of its weight from the 70th up to the 100th day, that of the normal only 20%.

From the fact that the initial progression of atrophy was practically the same in both prepuberally castrated and normal rats, it may be deduced that sex organs and sex maturity do not play any rôle in the process of physiological involution of the thymus.

[2] For these analyses we are indebted to Dr. J. M. Muñoz.

The height of the thymus weight curve was higher in the gonadectomized animals than in the normal, but the involution began at the same age. In previous works, only one point of the curve was studied, and as it was found that thymus weight was higher in the gonadectomized, it was deduced that the gonads determine physiological involution.

It should be noted that hypertrophy produced by castration during the adult age in most cases appeared to make the weight of the thymus equal to that of prepuberal castrates of the same age.

The growth curve of the lymphatic system of albino rats, as shown by the weight of the submaxillary, cervical and axillary groups of ganglions was different from that of the thymus, because the atrophic processes began later in both sexes (160 days of age), though it reached its highest peak at the same time as the thymus.

Prepuberal castration in both sexes gave rise to a small increase in weight of the ganglions. These findings may not apply to the whole of the lymphatic system, since only a part of it could be studied.

The decrease of thymic size, produced by injection of female sex hormone, estrone, or of male sex hormone, testosterone, cannot be attributed to traumatical or toxic effect of the solvent, since the controls, which were given injections of the diluent only, did not produce any changes of the organ. On the other hand, there were no important changes of body weight in experimental animals. Therefore, male and female sex hormones do actually produce a marked atrophy of the thymus. This effect is apparent in both sexes when an equal dose of the same hormone is given; the intensity of atrophy is similar in normal organs and in those where the thymus is hypertrophied consecutively to prepuberal castration. There exists a certain relationship between the dose and the effect produced.

It is to be expected that in the course of further experiments the physiological dose, that is, the quantity necessary to keep the thymus of a castrated animal on the level of normal development, will be determined.

Histological examination showed marked structural changes of the thymus, with increase of connective tissue; but this does not imply that only because of the conjunctive proliferation it ought to be included within accidental involution for adipose tissue was also very much increased. The latter process is considered as a typical element of physiological involution. The histological picture of the atrophy produced by either male or female hormones presents similar characteristics.

All that can be said here is that sex hormones, hydroxy and dihydroxy folliculin, testosterone propionate, independently of sex and presence or absence of sex organs, exert moderating or reducing trophic effects upon the thymus, the intensity being in proportion to the amount injected.

Sex hormones probably retard the growth of the thymus, maintaining it at a certain level. This would fully explain the higher weight curve of the thymus of rats castrated during the prepuberal stage and the increase due to castration during the adult stage.

Thyroidectomy caused a decrease in size of thymus already hypertrophied by prepuberal castration. This decrease is not due to a diminished body growth, for it was also found when considering thymus weight related to body weight. Moreover it was found that the thymus weight of animals in which thyroid regeneration took place was similar to that of the castrated ones, though in many instances body weight was hardly greater than that of castrated and thyroidectomized animals.

In rats castrated during the prepuberal period and then adrenalectomized at 80 and 120 days of age, there was only a slight increase of thymic weight as compared with that of the castrated rats.

SUMMARY

In male and female albino rats, castrated during their prepuberal period (30 days of age), the thymus was heavier than in the controls during the period between 45 and 250 days of age. The weight increase was due to an actual hypertrophy of the parenchyma. An evident increase was also produced in both sexes by adult castration.

The weight curve of the thymus of rats of either sex, castrated during the prepuberal period, was higher, but nearly parallel to that of the normal ones. The shape of the curve was not influenced by castration, therefore the physiological involution is not determined by the development of the sex glands.

No differences were evident histologically (either at the beginning, or in the moment of most intense physiological involution) between the thymus of animals castrated during prepuberal period and normal ones. Prepuberal castration produced a slight weight increase of submaxillary, cervical and axillary lymphatic ganglions of albino rats. Their involution began at 160 days of age in castrated as well as in normal animals, and therefore appeared later than the thymic involution.

Male hormone injection (testosterone propionate) provoked atrophy of thymic parenchyma of castrated as well as normal albino rats of either sex. Female hormone injection (estrone or estradiol benzoate) gave rise to atrophy of thymic parenchyma of normal and castrated albino rats of either sex. Histological changes produced by either hormone were intense atrophy of thymic lobules with extensive destruction of thymic lymphocytes and increase of connective and principally of fatty tissue.

Thyroidectomy decreased the size of thymus already hypertrophied as a result of prepuberal castration in both sexes. In female albino rats, castrated during prepuberal stage, adrenalectomy produced but a slight increase of hypertrophy determined by gonadectomy.

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THE OUTPUT OF PROTEIN METABOLISM HORMONE OF THE PITUITARY ANTERIOR LOBE

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IN PREVIOUS papers we have been able to adduce evidence that regulation of protein metabolism exists. We tested the regulation by a double administration of a protein test meal (gelatine). In normal persons a second administration 3 hours following the first either does not produce any rise of the amino-acid level of the blood, or at the most a very small rise (1). Further analysis revealed hormonal and central nervous influences (2, 3, 4). The regulating effect was found to be missing in cases of pituitary deficiency, pituitary cachexia, (3). This indicates that a factor of pituitary origin is involved in protein metabolism, and that tests of this factor may be made by the method of double administration of gelatine.

The fact that in normal cases the second administration of gelatine is not followed by a rise in the blood amino N could be explained as due to a regulation in which the anterior pituitary lobe is of first significance. It was assumed that protein feeding stimulates the anterior lobe to the production of the hormone active in protein metabolism. Experiments described in this paper were performed with the view of tracing and testing the output of this hormone.

EXPERIMENTAL

Normal men were given a carbohydrate-fat diet, practically free from protein, for 3 days. (A) Urine produced on the evening of the third day and on the early morning of the fourth day was collected. (B) The individual was then kept for 3 days on a diet consisting mainly of protein and urine was collected in the same way.

To test the presence of hormone we injected urine intraperitoneally into rats. Injection of extracts ot the anterior lobe, active in protein metabolism, have been shown to produce a marked decrease in blood nitrogen values within 6 to 8 hours (3, 5, 6, 7). If there was a considerable amount of the hormone active in protein metabolism, expected in the urine, injection of samples of this urine should consequently produce a decrease in the blood nitrogen values in rats.

Rats of about 200 gm. weight were fasted for 15 to 20 hours; water was given freely. Blood samples were obtained by heart puncture. The urine (A or B respectively) was injected intraperitoneally and 8 hours later blood was again withdrawn by heart puncture. Urea determination was performed by Rappaport's method (8). Amino N was determined by Folin's colorimetric method as modified by Rappaport (8). The experimental data of this series are given in table 1.

The decrease of blood urea nitrogen in 14 rats, injected with urine from 3 men on protein diet averaged 2.2 mg.% (4,5 and 5 rats used for each of the 3 urine samples, respectively). The reaction of the individual rats differed to some extent; 3 of the 15 showed a drop of urea nitrogen of less than 1 mg.% which is considered insignificant; in 1 of the 14 there was even a rise of blood urea nitrogen.

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In 13 rats injected with urine from the same three individuals on protein-free feeding there was no decrease of blood urea nitrogen (4, 4 and 5 rats used for each of the 3 urine samples respectively).

Blood amino N values were not determined in all animals of this series. There was no marked decrease of amino N in the rats injected with urine following protein feeding, the average drop in 7 rats being only 0.5 mg. %. The average drop in 6 rats injected with urine following protein-free feeding was 0.8 mg. %. Both of these values being below 1 mg. %, they are not considered significant.

Although it is evident that protein feeding leads to the output of some substance in the urine influencing protein metabolism, yet the effect of this substance does not correspond in every way with what is known of the effect of the anterior pituitary on protein metabolism. Extracts of pituitary anterior lobe are known to produce a decrease of both urea N and amino N in the blood of rats. The urine of the

TABLE 1

A. Injection: Urine following protein free feeding							B. Injection: Urine following protein feeding						
Rat No. Amount of urine injected	Urea N before injection	Urea N 8 hr. after injection	Decrease or increase	Amino N before injection	Amino N 8 hr. after injection	Decrease or increase	Rat No. Amount of urine injected	Urea N before injection	Urea N 8 hr. after injection	Decrease or increase	Amino N before injection	Amino N 8 hr. after injection	Decrease or increase
			Experiment 1							*Experiment 1*			
1 10 cc.	16.5	15.9	−0.6	9.4	7.8	−1.6	1 10 cc.	20.2	17.8	−2.4	7.4	6.9	−0.5
2 6 cc.	14.1	14.7	+0.6	7.6	9.6	+2.0	2 6 cc.	17.4	13.6	−3.8	8.5	7.3	−1.2
3 5 cc.	15.2	16.2	+1.0	7.7	7.2	−0.5	3 6 cc.	16.0	12.8	−3.2	—	—	—
4 5 cc.	12.3	12.1	−0.2	8.0	7.4	−0.6	4 10 cc.	19.8	18.1	−1.7	7.0	6.9	−0.1
(Average)	14.5	14.7	−0.2	8.2	8.0	−0.2	(Average)	18.3	15.6	−2.7	7.3	7.0	−0.3
			Experiment 2							*Experiment 2*			
1 10 cc.	9.7	9.0	−0.7	8.1	7.6	−0.5	1 8 cc.	15.3	12.2	−3.1	7.4	7.1	−0.3
2 6 cc.	12.4	13.1	+0.7	9.7	8.0	−1.7	2 8 cc.	17.2	16.9	−0.3	—	—	—
3 6 cc.	11.4	11.1	−0.3	—	—	—	3 8 cc.	12.2	10.2	−2.0	—	—	—
4 6 cc.	17.3	18.2	+0.9	—	—	—	4 8 cc.	13.8	13.4	−0.4	6.5	7.8	+1.3
(Average)	12.7	12.8	+0.1	9.9	7.6	−1.3	5 8 cc.	12.9	14.5	+1.6	7.5	7.3	−0.2
							(Average)	14.3	13.4	−0.9	7.1	7.4	+0.3
			Experiment 3							*Experiment 3*			
1 8 cc.	15.2	16.3	+1.1	—	—	—	1 10 cc.	15.7	11.3	−4.4	7.6	6.4	−1.2
2 10 cc.	14.7	13.8	−0.9	—	—	—	2 10 cc.	17.6	14.4	−3.2	—	—	—
3 10 cc.	16.2	16.8	+0.6	—	—	—	3 8 cc.	20.6	16.7	−3.9	—	—	—
4 6 cc.	11.4	10.9	−0.5	—	—	—	4 8 cc.	18.7	15.3	−3.4	—	—	—
5 10 cc.	12.6	13.2	+0.6	—	—	—	5 10 cc.	17.0	16.3	−0.7	—	—	—
(Average)	14.0	14.2	+0.2	—	—	—	(Average)	17.9	14.8	−3.1	7.6	6.4	−1.2
Average of 3 experiments	13.7	13.9	+0.2	8.6	7.8	−0.8	Average of 3 experiments	16.7	14.5	−2.2	7.4	6.9	−0.5

individuals on protein feeding produced only a decrease of urea N, but did not change amino N values.

There are two possible explanations for this difference of action of pituitary extracts and of the substance excreted in the urine during our experiments. The first is that the factor or hormone influencing urea N level and the factor or hormone influencing amino N level might not be the same. Protein feeding would then only produce output of the factor responsible for changes of urea N, whereas in pituitary extracts both the factor responsible for urea N changes and for amino N changes would be present. We did not consider this to be in any way probable. Secondly, there is the possibility that the quantity of hormone necessary to bring about a decrease of amino N might be greater than the quantity necessary to decrease urea N.

The anterior pituitary spurs protein anabolism, utilizing for protein-synthesis the amino acids available and thus producing a drop of amino N in blood and tissues. Drop of urea N in this process is merely the consequence of the fact that less material for deamination and urea formation is available.

If deamination of amino acids actually available at the beginning of the experiment were inhibited by the anterior pituitary hormone, if in other words the pitui-

tary hormone were influencing the 'lower stage' of protein metabolism to some extent independently from its action on the 'upper stage' (in terms of Neubauer, 9), then inhibition of deamination and urea formation would primarily have a tendency of increasing the amino N level of the blood. The final decrease of the amino N would only be produced by a larger amount of hormone, influencing the 'upper stage' of protein metabolism, viz. the synthesis of protein from amino acids. Drop of urea N would appear more readily because both the diminished deamination ('lower stage'-process) and the increased protein-synthesis ('upper stage'-process) must necessarily decrease urea N level.

Acting according to this hypothesis we tried to increase the amount of urine injected into the test rats. As it seemed hardly advisable to inject a much larger quantity of urine, we made preparations of the urine samples in the following way.

TABLE 2

A. Injection: Urine concentrates following protein free feeding							B. Injection: Urine concentrates following protein feeding						
Rat No. Amount of urine-concentrate injected	Urea N (mg. %) before injection	Urea N (mg. %) 8 hr. after injection	Decrease or increase	Amino N (mg. %) before injection	Amino N (mg. %) 8 hr. after injection	Decrease or increase	Rat No. Amount of urine-concentrate injected	Urea N (mg. %) before injection	Urea N (mg. %) 8 hr. after injection	Decrease or increase	Amino N (mg. %) before injection	Amino N (mg. %) 8 hr. after injection	Decrease or increase
		Experiment 4							*Experiment 4*				
1 10 cc. (=50 cc. urine)	17.8	16.9	−0.9	6.6	7.5	+0.9	1 10 cc. (=50 cc. urine)	18.7	14.1	−4.6	9.3	6.6	−2.7
2 10 cc. (=50 cc. urine)	17.2	16.3	−0.9	6.9	5.7	−1.2	2 10 cc. (=50 cc. urine)	16.5	14.5	−2.0	9.3	6.7	−2.6
3 8 cc. (=40 cc. urine)	15.8	15.4	−0.4	6.5	6.4	−0.1	(Average)	17.6	14.3	−3.3	9.3	6.6	−2.7
(Average)	16.9	16.2	−0.7	6.7	6.5	−0.2							
		Experiment 5							*Experiment 5*				
1 8 cc. (=40 cc. urine)	16.6	17.6	+1.0	7.5	6.0	−1.5	1 8 cc. (=40 cc. urine)	20.1	19.4	−0.7	9.4	14.0	+4.6
2 8 cc. (=40 cc. urine)	18.5	17.6	−0.9	6.4	10.9	+4.5	2 8 cc. (=40 cc. urine)	—	—	—	10.2	8.0	−2.2
3 8 cc. (=40 cc. urine)	—	—	—	6.4	9.4	+3.0	3 8 cc. (=40 cc. urine)	18.7	14.5	−4.2	11.3	7.9	−3.4
4 8 cc. (=40 cc. urine)	16.8	15.7	−1.1	7.5	14.3	+6.8	4 8 cc. (40 cc. urine)	18.2	12.3	−5.9	9.5	7.8	−1.7
(Average)	17.3	16.9	−0.4	7.0	10.1	+3.1	(Average)	19.0	15.4	−3.6	10.1	9.4	−0.7
Average of both experiments	17.1	16.6	−0.5	6.8	8.3	+1.5	Average of both experiments	18.3	14.8	−3.5	9.7	8.0	−1.7

Urine samples were collected in the same way as in the first series after 3 days of protein feeding and after 3 days of protein-free carbohydrate-fat feeding, respectively. One volume of urine was mixed with 5 volumes of 95% alcohol, the mixture was thoroughly stirred, centrifuged and the precipitate taken up in distilled water in quantities corresponding to 1/5 of the original quantity of urine. The precipitate did not completely dissolve in water but gave a milky emulsion. Using this emulsion for intraperitoneal injection into rats we were able to administer, per unit of fluid, 5 times the amount of substances precipitated by alcohol which are present in the original urine sample. Otherwise the experiments in this second series were performed in exactly the same way as in the first.

The data of this series are given in table 2. The decrease of urea N was more marked than in the first series, the average drop in 5 rats injected with concentrated urine precipitates from 2 men after protein feeding being 3.5 mg. %. There was practically no decrease of urea N in the rats injected with concentrated urine precipitates following protein-free feeding.

In this series there was a marked decrease in amino N. The average drop in 6 rats injected with urine preparation from 2 men following protein feeding was 1.7 mg. %, even though, for unexplained reasons, one of the 6 rats showed a rise of as much as 4.6 mg. % amino N (2 and 4 rats used for each urine sample respectively).

If we exclude this animal, the average drop of amino N was 3.8 mg. %. The 7 rats injected with concentrated urine preparations following protein-free feeding showed an average increase of amino N of 1.5 mg. % (3 and 4 rats used for each urine sample respectively).

DISCUSSION

In human urine collected after a period of protein feeding, it was possible to trace a factor active in protein metabolism. Injection of these urine samples or of concentrated urinary precipitates into rats produced a decrease of blood urea N and of blood amino N. This effect was missing when urine samples, collected after a period of protein-free diet, were used for injection.

The experiments were performed on males only because we wished to exclude the possible influence of estrual changes on the output of the factor active in protein metabolism. The possibility of such an influence was suggested by the results of one experiment performed on a female. This experiment is not recorded in this paper.

Attempts to trace the factor in human blood after a protein meal have been made and have been briefly reported (3). These experiments were not successful. Influence of the proteins of the injected blood on the blood nitrogen values of the test rats could not be excluded.

The reaction in the rat produced by the injection of urine collected after protein feeding closely resembles the reaction produced by injection of alkaline pituitary extracts. Pituitary extracts produce a decrease of non protein nitrogen, urea N and amino N in rats, dogs and guinea pigs (3, 5, 6, 7). This drop of non protein nitrogen values of the blood is not due to increased urinary excretion; on the contrary nitrogen output decreases after injection of pituitary extracts (7). At the same time amino N and urea N values decrease in tissues (6, 10, 11). The conclusion drawn from these experiments is that pituitary extracts spur protein anabolism and the synthesis of protein from amino acids.

In the experiments reported in this paper the nitrogen excretion of the test rats was not examined, neither were tissue analyses performed. But in so far as blood urea and blood amino N values are concerned, the urine samples after protein feeding produced the same changes as do pituitary extracts.

It has been reported previously that administration of a protein test meal brings into action a factor which prevents a rise of blood amino N after a second protein meal administered 3 hours after the first (1). This factor was found to be missing in cases of pituitary deficiency. Hence it was concluded that this factor is of pituitary origin (3).

A factor active in protein metabolism is present in alkaline pituitary extracts. A factor of pituitary origin active in protein metabolism has been detected by double administration of protein. A factor active in protein metabolism has now been found in urine following protein intake, the latter factor producing the same effect in test rats as do pituitary extracts. It seems highly probable that these factors, traced by three different experimental proceedings are one and the same and that it is of pituitary origin.

Much evidence had been adduced that the growth hormone of the pituitary anterior lobe is the hormone influencing protein metabolism (7). Extracts found active in protein metabolism were alkaline extracts containing growth hormone. True growth obviously must involve building up of proteins. It has been experimentally shown that growth and composition of tissues following administration of growth hormone are closely related and parallel (6, 10, 11). All this speaks strongly in favor of identity of growth hormone and protein metabolism hormone. On the other hand,

in cases of pituitary nanosomia double administration of a protein meal was found to produce a normal effect on blood amino acids, viz. no rise of blood amino N occurred after the second protein meal. The protein metabolism hormone was present (2). In view of the fact that in pituitary dwarfism we have to assume a permanent lack of growth hormone, it was concluded that protein metabolism hormone and growth hormone could not be identical. It was suggested that possibly in alkaline pituitary extracts both growth hormone and protein metabolism hormone were extracted simultaneously. We do not consider this problem to be definitely solved.

In the experiments described in this paper smaller doses of active urine producing a marked effect on blood urea N of rats, failed to influence the amino N. Only after increasing the dosage were we able to produce a decrease of amino N as well as of urea N. The decrease of urea N as well as amino N due to the action of the anterior pituitary is generally understood to be the consequence of protein anabolism. This process is bound to produce decrease of free amino acids, because they are utilized in building up protein. Decrease of urea is due to the lack of amino acids available for deamination. A difference in the amount of hormone necessary to influence urea N and amino N cannot occur within this process. The difference of amount of hormone necessary for decreasing urea and amino acids suggests an action of the hormone on deamination as well as on anabolism. If deamination of amino acids is inhibited by the hormone this would produce a decrease of urea N only, whereas amino N might even show a tendency to increase. Larger doses of hormone would produce full action, not only diminishing deamination, but also synthesizing protein. Urea formation would be diminished by both the action of the hormone on deamination and on synthesis of protein, whereas amino acids would have a tendency to increase by the hormonal influence on deamination and to decrease by the influence on protein synthesis. This hypothesis would explain the results of the experiments.

The fact that protein feeding is an adequate stimulus for the production of a hormone anabolising protein is in accordance with our knowledge of carbohydrate metabolism. Carbohydrate feeding is the adequate stimulus for the output of insulin, the anabolic hormone of carbohydrate metabolism. A mode of regulation probably very similar in fat metabolism has been described (12). Regulation of metabolism of inorganic substances also seems to be based on the same principle as can be deduced from experiments on calcium metabolism (13, 14). Our findings in protein metabolism apparently exemplify a general physiological rule.

SUMMARY

Protein feeding is followed by the output of a factor in the urine which, when injected into rats, produces a decrease of blood urea N and of blood amino N. This factor is not present in the urine after a protein-free carbohydrate-fat diet. This reaction to injection of urine following protein intake (decrease of non-protein nitrogen constituents in the rats) is the same as is obtained by injecting alkaline extracts of the pituitary. It is highly probable that the factor traced in the urine after protein feeding is of pituitary origin.

As has been previously shown a factor of pituitary origin also prevents rise of blood amino N after a protein meal given 3 hours after a similar previous protein meal. Protein feeding is the adequate stimulus for the output of this hormone.

Stimulation of the output of hormone by administering the substance on which the hormone specifically acts is not peculiar to protein metabolism, and we may therefore assume that this mechanism represents a general physiological rule.

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THE EFFECT OF ESTRIN ON THE ANTERIOR PITUITARY OF MALE RATS AFTER PITUITARY STALK SECTION

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THE REGULATION of the gonadotropic function of the hypophysis is at present only partially understood. As far as nervous regulation is concerned, the rôle of the cervical sympathetics seems to be of minor importance as compared with the hypothalamo-hypophyseal pathways (1, 2, 3). It might be mentioned here that Brooks (4) found that pituitary stalk section prevented ovulation after mating in rabbits, although the gonads appeared to be histologically normal. Dempsey (5), on the other hand, showed that in guinea pigs not even ovulation was disturbed by stalk section, while Uotila (3) noted that in male rats the gonads remained normal after this operation, although the depressing effect of cold on spermatogenesis was prevented.

Fluctuations of the level of circulating sex hormones also affect the gonadotropic function of the hypophysis, either through the hypothalamo-hypophyseal system or directly from the circulating blood. This problem was opened up by Hohlweg and Junkmann (6) who transplanted hypophyseal tissue into the kidneys of castrated rats and noted that 'castration cells' did not appear in the transplant, although they could be seen in the animal's own intact pituitary. They also observed that the castration cells disappeared from a castration pituitary which is transplanted into the kidneys of a castrated host. On the basis of these experiments they advanced the theory that there exists in the hypothalamus a hypothetical 'sexual center' which stimulates the production of gonadotropic hormones in castrated animals. They further assumed that the depressing effect of the sex hormones on the gonadotropic hormones is mediated through this 'sexual center.' In the face of experiments by Desclin and Grégoire (7), Hohlweg (8) was forced to modify the theory and admit that sex hormones might depress the gonadotropic function directly through the anterior pituitary.

Desclin and Grégoire (7) noticed the absence of castration cells in pituitary tissue grafted into the kidneys, but they emphasized certain changes in the basophil cells of the transplant which they interpreted as a possible reaction to castration or to the unphysiological environment of the transplant. Furthermore, they described cytological changes in the graft after massive estrin treatment, from which they concluded that the graft was probably not sensitive enough to react to small depressions of sex hormone levels, although marked elevations in estrin titre would produce noticeable effects. From the study of transplants introduced into hypophysectomized rats Martins (9) and Phelps, Ellison and Burch (10) concluded that, although a hypophyseal graft reacts cytologically to estrin, its gonadotropic and other functions are impaired. Haterius (11) was able to demonstrate castration cells in pituitary tissue transplanted to the eye and kidney of castrated animals. Westman and Jacobsohn (12) have recently pointed out that after pituitary stalk section and castration, castration cells are completely absent, although the hypophysis reacts to estrin injections by displaying increased size and certain cytological changes.

[1] Fellow of the Rockefeller Foundation. This study was partially aided by a grant from the Josiah Macy, Jr., Foundation.

Although many other observations suggest indirectly that quantitative variations in sex hormone levels may affect the anterior pituitary without nervous mediation, it is obvious that numerous aspects of this problem require further clarification.

The present study is based on the fact that estrin, when injected into male rats, acts through the anterior pituitary to produce an atrophy of the testicles and seminal vesicles (13, 14). If Hohlweg and Junkmann are correct in their theory, estrin injections should not cause gonad atrophy after stalk section. If, on the other hand, estrin affects the anterior pituitary without any mediation through hypothalamic sexual centers, and directly suppresses the production of gonadotropic hormones, pituitary stalk section should not interfere with this action. Finally, attention was paid to the possibility that, although estrin affects the gonadotropic function directly, the cutting of the stalk might modify these direct effects of estrin.

This paper presents the results of an investigation of these three possibilities. Two groups of rats were treated with identical amounts of estrin. The first group of animals with severed pituitary stalks was compared with the second group with intact stalks. The effect of estrin injection on the size and cytological picture of the anterior pituitary was noted, and the weights and histological appearance of the testes and seminal vesicles were used as indications of gonadotropic secretion. The changes in the adrenals and the thyroids were also followed.

MATERIAL AND METHOD

Twenty-two male albino rats of Wistar strain, weighing 130–150 gm. at the time of operation, were used as experimental animals. The number of animals used in each group and the experimental procedures are recorded in the accompanying table 1.

Stalk sections were performed using a method which has been described in a previous paper (3). After the operation the development of the animals was observed for 30 days, and all the cases with complications or signs of pituitary insufficiency, such as delayed growth or gonadal atrophy, were discarded.

The estrin treatment consisted of daily injections of 20 R.U. of estradiol benzoate (progynon-B, Schering Corp.) for 20 days.[2]

At the end of the experiment all of the rats were killed and the testicles, seminal vesicles, adrenals and thyroids were weighed and fixed in 10% formol for histological studies. The skulls were fixed in formol and decalcified in 5% trichloracetic acid. Serial sections of the hypothalamo-hypophyseal region were prepared in order to check the completeness of stalk section. Hematoxylin and eosin were used to stain all of the organs excepting the hypophysis. It was found that the Koneff (15) modification of the Mallory-azan stain gave the best results with the pituitary. This staining method gives a consistently good differentiation of the various types of cells in the anterior pituitary. The quantitative data were handled statistically.

RESULTS

The effect of estrin in normal male rats. The injection of 20 R.U. daily of estrin for 20 days stopped the weight increase of our male rats for the duration of the experiment (table 1).

(*a*) *Hypophysis.* The size of the anterior pituitary was greatly increased by the estrin injections (about two times the normal diameter), and the gland was hyperemic. Histological examination showed that the basophils were degranulated and that

[2] The author is grateful to Dr. M. Gilbert of the Schering Corporation of Bloomfield, New Jersey, for his generosity in supplying the estrin used in this study.

their number was relatively decreased. The acidophils also showed degranulation, and many displayed large, clear, peripheral, cytoplasmic vacuoles. In certain parts of the gland these cells were degenerating. One got a general impression that the relative number of acidophils had decreased, although no cell counts were made. Many chromophobe cells appeared larger than those in uninjected controls, and some were vacuolated. These changes were variable, but were most prominent in the largest pituitaries. In those pituitaries which showed the least enlargement the acidophils seemed to be increased in number instead of decreased as in the larger glands.

(*b*) *Testicles.* The relative mean weight of the testes in injected animals was decreased by 39.4% when compared with the corresponding value of uninjected controls (table 1). The tubules usually showed very marked atrophic changes: spermatogenesis had ceased and the spermatogonia and spermatocytes were partially or totally atrophied, the degree of atrophy varying somewhat from place to place.

TABLE 1. THE EFFECT OF ESTRIN ON THE VARIOUS ENDOCRINES IN INTACT AND STALK-CUT ANIMALS

Treatment	Mean weight of organs in mg./gm. of body weight following standard deviation					Mean body weight in gm. with standard deviation	
	No. rats	Thyroid	Adrenals	Testes	Seminal vesicles	Beginning experiment	End of experiment
Normal, unoperated controls.	6	0.067± 0.0009	0.102± 0.0014	10.89± 0.49	1.410− 0.078	228.2± 2.2	292.3± 8.3
Unoperated rats, treated with estrin.	9	0.078± 0.0053	0.188± 0.010	6.601± 0.84	0.597± 0.031	207.8± 3.3	210.4± 6.4
Complete pituitary stalk section and estrin treatment.	6	0.082± 0.0043	0.185± 0.043	6.520± 0.32	0.567± 0.051	209.2± 7.1	207.5± 6.5
Incomplete pituitary stalk section and estrin treatment.	1	0.074	0.175	7.373	0.600	217.0	217.0

(*c*) *Seminal vesicles.* The weight loss of the seminal vesicles in estrin-treated rats averaged 57.7% when compared with uninjected controls (table 1). This decrease in weight seemed to result from atrophic changes in the secretory epithelium, which was cuboidal or even flat, instead of columnar as in controls. There was no evidence of mucous secretion or of secretory granules. Such marked degeneration in the seminal vesicles was noted even when the testicular atrophy was relatively slight.

(*d*) *Adrenals.* The mean relative adrenal weight in injected animals was 84.3% above that of uninjected controls (table 1). This increase in weight was apparently a manifestation of general cortical hypertrophy, although in some cases the inner cortical layers were hypertrophied more than the outer.

(*e*) *Thyroid.* A statistically significant increase (+16.4%) was noted in the mean relative weight of the thyroid glands of estrin injected rats when compared with controls, although the absolute weights were somewhat decreased (table 1). Histologically the glands seemed to be for the most part within normal limits, although occasionally one met with a flattening of the epithelium.

The effect of estrin after pituitary stalk section. After stalk section the growth of the animals and size of the testicles were followed for 30 days before estrin treat-

ment was begun. As reported previously (3), the growth curve is not significantly affected by the operation (table 1). The size of the testicles remained normal after stalk section until estrin treatment was begun.

Estrin treatment after stalk section produced the same cessation of weight increment noted in the series of rats receiving estrin with stalks intact (table 1).

(*a*) *Hypophysis.* The size of the pituitaries was greatly increased in stalk-sectioned, estrin-treated rats, as in the estrin-treated controls. The pituitaries also showed the same degranulation of basophils and acidophils, vacuolization and degeneration of acidophils, as well as the same increase in chromophobes as in the controls. One animal with an incomplete stalk section displayed identical changes after estrin treatment.

(*b*) *Testicles.* In the estrin-treated, stalk-sectioned rats the mean relative weight of the testicles had decreased on the average by 40.1% as compared with 39.4% decrease in estrin-treated animals with stalk intact (table 1). A tubular atrophy, as marked as that produced in estrin-treated animals with intact stalk, was noted in the stalk-sectioned series.

(*c*) *Seminal vesicles.* The mean weights of the seminal vesicles in the present series of rats were decreased by 59.8% as compared with a 57.7% decrease in estrin-treated animals with intact stalks (table 1). Both groups showed histologically the same marked atrophic changes.

(*d*) *Adrenals.* An average weight increase of 81.3% was noted in the adrenals of this group of rats (table 1), and the histological picture of cortical hypertrophy was apparent, just as in the series of intact stalk, estrin-injected rats.

(*e*) *Thyroid.* A statistically significant increase of 22.3% in the mean relative weight of the thyroid gland was noted in this series (*vs.* 16.4% in estrin controls). The glands were histologically within normal limits, although the epithelium showed a tendency to become flattened.

DISCUSSION

Hypophysis. These experiments show that estrin treatment produces a hypertrophy of the anterior pituitary to an equal extent in animals with the stalk intact and with the stalk cut. In both groups the hypertrophy is associated with a partial degranulation, vacuolization and partial degeneration of the acidophils, whereas the basophils become degranulated. An increase in the chromophobes occurs, resulting in the appearance of numerous large clear cells. These cellular changes have been noted previously to be characteristic of the effect of estrin injections in male rats (16). Hence, as Westman and Jacobsohn (12) have pointed out, stalk section does not affect the influence of estrin on the cytological picture of the hypophysis.

Testicles and seminal vesicles. Estrin treatment causes an equally marked atrophy of the testicles and seminal vesicles in operated and unoperated rats. These observations demonstrate that estrin affects the pituitary gonadotropic hormones without any mediation of the pituitary stalk. Since estrin causes changes in the cytological picture of the anterior lobe in pituitary transplants (7, 9, 10), the cervical sympathetics may also be ruled out as a possible mediator of estrin effects. From the present experiments, as well as from those just quoted, it may be safely concluded that estrin affects the pituitary directly. Conversely, it can be stated that estrin does not modify the direct effect of estrin on the gonadotropic function through hypothalamo-hypophyseal pathways.

Deficiencies of sex hormones resulting from castration and overdoses of sex hormones artificially administered differ from each other only quantitatively. For this reason it is difficult to believe that the influence of castration would be exerted

through the hypothalamo-hypophyseal system, whereas in a state of sex hormone excess the effect would be directly on the hypophysis. As mentioned in the introduction, Desclin and Grégoire (7), and Haterius (11) reached conclusions completely or in part at variance with those of Hohlweg and Junkmann (6). However, transplantation experiments are scarcely an ideal way to clarify this problem. Westman and Jacobsohn's (12) observation that castration cells are absent after pituitary stalk section and castration is of greater significance. Nevertheless, their results must be accepted with reservations until confirmed, because their operative technic produced cessation of growth and gonadal atrophy—findings which indicate anterior pituitary deficiency. Such evidence of insufficiency is not met with after stalk section when performed according to the technic used by Brooks (4), Dempsey (5) or Uotila (3).

Adrenals. An overdose of estrin causes a hypertrophy of the adrenals to an equal extent whether the pituitary stalk is intact or severed. The adrenal hypertrophy induced by estrin is mediated through the anterior pituitary (16). In another paper (3) it was reported that the hypertrophy of the adrenals produced by cold is also independent of the integrity of the stalk. These observations indicate that the corticotropic function of the anterior pituitary is humorally controlled.

Thyroid. As a result of estrin treatment there is a statistically significant increase in the weight of the thyroid per gram of body weight in intact animals, as well as after stalk section, although the absolute weights of the glands are decreased. However, since the histological findings are essentially normal, no conclusions can be reached on the effect of estrin on thyroid function.

General considerations. There is an increasing body of evidence indicating that the anterior pituitary has a basic secretory rhythm which is humorally controlled by the hormones of other endocrine glands. The present experiments confirm this conclusion in the case of the effect of estrin on the gonadotropic and corticotropic function in male rats. On the other hand, some anterior pituitary factors, such as luteinizing hormone in rabbits (4) and thyrotropic and gametogenic hormone in rats (3) may, under certain circumstances, be influenced by the hypothalamus. Only further detailed study, using various experimental procedures, can solve the question of what hormones and conditions are involved when the hypothalamo-hypophyseal system participates in the regulation of the anterior pituitary.

SUMMARY

Large doses of estrin injected into male rats act on the anterior pituitary without the mediation or any modifying effect of the pituitary stalk. This is evidenced by the following facts: (*a*) the anterior pituitary undergoes hypertrophy and shows the same cytological changes in rats which have received estrin whether the stalk be intact or severed; (*b*) estrin suppresses the secretion of gonadotropic hormones in stalk-cut and intact male rats to an equal extent, as shown by the appearance of a marked atrophy of the testicles and seminal vesicles in both groups.

The adrenals hypertrophy equally after estrin treatment in intact and stalk-sectioned animals, indicating that estrin also affects the corticotropic function of the anterior hypophysis without any mediation or modification of the pituitary stalk.

It is concluded that estrin acts directly on the anterior hypophysis, and that this action is a part of the humoral control of the basic secretory rhythm of the gland.

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THE REGULATION OF THYROTROPIC FUNCTION BY THYROXIN AFTER PITUITARY STALK SECTION

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THIS PAPER reports part of a series of experiments designed to clarify the mechanisms regulating the secretion of thyrotropin. More specifically, it seeks to answer the question as to whether fluctuations in an animal's thyroxin level affect the secretion of thyrotropin by direct humoral access or through the mediation of the hypothalamo-hypophyseal system.

When considering the regulation of thyrotropic function, at least three possible mechanisms must be taken into account. These possibilities are: (*a*) nervous influences through the cervical sympathetics; (*b*) impulses passing through the hypothalamo-hypophyseal stalk and (*c*) direct humoral influences.

Evidence has previously been presented indicating that cervical sympathectomy can slightly and temporarily decrease thyrotropic function, although the sympathetics are not necessary for continued normal thyroid function (1).

Subsequently the rôle of the hypothalamo-hypophyseal pathways was studied in reference to the regulation of thyrotropic function. It was shown that, although the thyroid remains histologically normal after pituitary stalk section, this operation prevents the normal hypertrophic reaction of the thyroid to cold. These experiments demonstrated that under certain circumstances the hypothalamo-hypophyseal system may transmit impulses influencing the secretion of thyrotropin (2, 3).

Since the thyrotropic function seemed to be undisturbed under ordinary environmental conditions in animals where cervical sympathectomy or pituitary stalk section was done, it was assumed that a direct humoral influence must be exerted upon the thyrotropic function of the anterior pituitary.

The basis of the present experiments is the fact that variations of thyroxin level affect thyrotropin secretion. Thyroxin treatment causes cytological changes in the anterior pituitary (4), and decreases the amount of thyrotropin in the anterior pituitary with resulting thyroid atrophy, according to the studies of Kuschinsky (5) and of Hohlweg and Junkmann (6). On the other hand, subtotal thyroidectomy causes cytological changes in the anterior pituitary (4) and compensatory hyperplasia of the residual thyroid tissue. Houssay et al. (7) and Kahler (8) showed that the compensatory hyperplasia is absent after hypophysectomy. After thyroidectomy or in myxedema the urinary thyrotropin is increased (9–12).

However, the mechanism of these effects is unknown. Schittenhelm (13) claims that the iodine content of the hypothalamus is relatively high. Pick (14) mentions that the so-called diencephalic brain narcotics decrease the toxicity of thyroxin, and Fenz (15) postulates a beneficial effect of these narcotics in thyrotoxicosis. On the basis of these observations Lichtwitz (16) has assumed that thyroxin regulates the thyrotropic function through the hypothalamo-hypophyseal pathways.

In the present paper it has been postulated that if an excess or deficit of thyroxin

[1] Fellow of the Rockefeller Foundation. This study was partially aided by a grant from the Josiah Macy, Jr. Foundation.

affects the thyrotropic function through the hypothalamo-hypophyseal pathways. pituitary stalk section should prevent the thyroid atrophy or compensatory hyperplasia (as the case might be) which would otherwise normally follow variations in thyroxin level. If the hypothalamo-hypophyseal system plays no rôle in the action of thyroxin on thyrotropin secretion, stalk section should not interfere with the influence of thyroxin. The possibility has also been kept in mind that thyroxin, besides acting on the anterior pituitary, may be influenced in its action by modifying effects mediated through the pituitary stalk.

In one group of unoperated rats (Section A) the effects of thyroxin on the thyroid and the other endocrine glands were observed. The results were compared with those obtained in a second group of rats (Sections *B* and *C*) given identical thyroxin treatment after stalk section. Finally a third group of rats (Section *D*) was observed in order to ascertain whether the normal compensatory hypertrophy of residual thyroid following subtotal thyroidectomy is present after pituitary stalk section.

MATERIAL AND METHODS

Thirty-three male albino rats of Wistar strain weighing 130 to 150 gm. at the time of operation were used as experimental animals. The number of animals used in each group and the experimental procedures followed are recorded in tables 1 and 2.

The method of pituitary stalk section has been described previously (3). The post-operative course of all of the rats was observed for 30 days, and any animals showing post-operative complications or signs of pituitary insufficiency were discarded. The thyroxin treatment consisted of daily intramuscular injections of 1 mg. of crystalline thyroxin (Hoffmann-LaRoche) per 200 gm. of body weight (5 mg. per kg.) for 10 days. The thyroxin was dissolved in N/100 KOH, 2 mg. of thyroxin to 1 cc. KOH solution. Control animals received an equivalent amount of N/100 KOH.

In the series of subtotally thyroidectomized rats, the whole left thyroid lobe and half of the right lobe were removed (3/4 thyroidectomy) and preserved in formol for comparison with the residual thyroid tissue. The animals were killed 7 days after subtotal thyroidectomy.

When the rats were autopsied, the thyroid, adrenals, testicles and seminal vesicles were removed, weighed and fixed in 10% formol for histological study. The base of the skull was also fixed in formol and subsequently decalcified in 5% trichloracetic acid for serial sectioning of the hypothalamo-hypophyseal region in order to check the completeness of the stalk section. Out of 14 successfully operated animals 4 proved to have sustained an incomplete stalk section and 10 were complete. The hypophyses were stained by Koneff's (17) modification of the Mallory-azan stain, while all the other glands were stained with hematoxylin and eosin.

The Cell Height Index (CHI) was used as an indicator of thyroid activity. The CHI equals the average height (in μ) of the cells in 100 thyroid follicles counted in a systematic way. From each follicle a cell which seemed to represent the mean height of all the cells in that follicle was chosen and measured (1).

All quantitative data were statistically handled, the mean values and the standard deviation were calculated, and in equivocal instances the statistical significance of the findings was recorded.

RESULTS

A. Effect of Thyroxin in Rats with Intact Pituitary Stalk

The mean weight loss in this group of animals was 13.9%.

Hypophysis. The acidophiles were somewhat smaller in size and stained more densely than in normal rats. There was a slight tendency toward an increased number of acidophiles, though this was not a constant finding. The basophiles were distinctly increased in number and in size. A few vacuolated basophiles were seen, but these were also occasionally found in controls.

Thyroid. The mean relative weight of the thyroids was somewhat smaller than in normal controls, but the difference is not significant (table 1). The CHI was 41.6% smaller than in normal controls (table 2). The value for the CHI (2.26), after 10 days of thyroxin treatment, was about the same as in animals which had been hypophysectomized for 10 days (1).

Adrenals. The mean relative weight of the adrenals was 38.4% larger than in controls (table 1). Histological study revealed a general cortical hypertrophy combined in some instances with a diminution in the cortical lipin vacuoles.

Testicles. The mean relative weight of the testicles was not affected by the thyroxin treatment (table 1). In most cases the histological picture was normal,

TABLE 1. THE EFFECT OF THYROXIN ON VARIOUS ENDOCRINES AND ON BODY WEIGHT IN INTACT ANIMALS AND AFTER PITUITARY STALK SECTION

Treatment	No. rats	Weight of organs in mg. per gm. of body weight accompanied by standard deviation[1]				Body weight of animals in gm. with standard deviation[1]	
		Thyroid	Adrenals	Testicles	Seminal vesicles	At the beginning	At the end
Normal untreated controls	9	0.0754± 0.0034	0.146± 0.0065	11.730± 0.47	1.326± 0.053	221.1± 6.3	220.2± 6.8
Unoperated rats, treated with thyroxin	10	0.0698± 0.0032	0.202± 0.0064	11.527± 0.107	0.797± 0.061	211.3± 4.0	182.2± 4.2
Complete pituitary stalk section and thyroxin treatment	6	0.0839± 0.0054	0.194± 0.0075	11.271± 0.62	0.912± 0.062	204.7± 6.4	172.8± 6.5
Incomplete pituitary stalk section and thyroxin treatment	4	0.0661± 0.032	0.166± 0.055	11.065± 0.823	0.939± 0.035	219.7± 11.5	189.3± 5.5

[1] Because of the limited amount of material the so-called safe standard deviation has been used.

although sometimes the tubules were somewhat smaller and contained fewer spermatocytes than the controls.

Seminal vesicles. As a result of thyroxin treatment a weight loss of 39.9% took place in the seminal vesicles. This seemed to be accompanied by decreased secretory activity, as evidenced by the absence of mucous secretory stages and secretory granules. Although the epithelium in the vesicles of injected animals was usually lower than in controls, the majority showed evidence of some secretory activity.

B. The Effect of Thyroxin after Complete Pituitary Stalk Section

The mean loss of body weight in this group was 15.6% as compared with a loss of 13.9% in animals with intact stalk after thyroxin treatment.

Hypophysis. The changes seen after thyroxin injection in animals with severed stalks were similar to those seen after thyroxin injection in animals with intact stalks. More specifically, one could also note in this group increased numbers of acidophiles more darkly staining than in controls. These cells were somewhat decreased in size. The basophiles were irregularly increased in size, some of them appearing vacuolated.

Thyroid. The mean relative weight of the thyroid was somewhat larger than in normal controls or in injected animals with intact stalks, though the difference was not significant (table 1). The decrease in CHI was 44.7% as compared with a decrease of 41.6% after thyroxin treatment alone (table 2). In this series also the CHI of 2.14 after 10-day thyroxin treatment was comparable to the CHI 10 days after complete hypophysectomy (1).

Adrenals. The increase in mean relative weight was 32.9% (38.4% in thyroxin controls), and the same cortical hypertrophy and partial loss of lipin vacuoles were found as in thyroxin controls.

Testicles. The mean relative weight was normal. The histological picture showed the same characteristics as after thyroxin treatment alone.

Seminal vesicles. The weight loss of the seminal vesicles in this group was 31.3% against 39.9% in thyroxin controls, and the epithelium showed the same evidence of damaged secretory activity.

TABLE 2. THE EFFECT OF THYROXIN AND SUBTOTAL THYROIDECTOMY ON THE CELL HEIGHT OF THYROID AFTER PITUITARY STALK SECTION

Treatment	No. rats	CHI with standard deviation[1]	Treatment	Rat no.	CHI before ¾ thyroidectomy	CHI after ¾ thyroidectomy	Difference
							%
Normal untreated controls	9	3.87± 0.36	Complete pituitary stalk section and ¾ thyroidectomy for seven days	561	3.12	4.12	+32.1
Unoperated rats treated with thyroxin	10	2.26± 0.02		588	2.97	4.30	+44.5
Complete pituitary stalk section and thyroxin	6	2.14± 0.05		590	3.18	3.90	+22.6
Incomplete pituitary stalk section and thyroxin	4	2.17± 0.04		609	3.64	4.92	+35.1
				Av.	3.23	4.31	+33.5

[1] Because of the limited amount of material the so-called safe standard deviation has been used.

C. The Effect of Thyroxin after Incomplete Pituitary Stalk Section

Four rats in which pituitary stalk section was attempted proved on examination to have sustained severe damage to the stalk without its being completely severed. As seen in tables 1 and 2, the quantitative data obtained from the various endocrines after thyroxin treatment are essentially the same whether the stalk is intact or partially or completely interrupted. This is also true regarding the effect of thyroxin on the cytological appearance of the anterior pituitary, adrenals and seminal vesicles.

D. The Effect of Subtotal Thyroidectomy on the Thyroid and Other Endocrines after Complete Stalk Section

Hypophysis. Two out of 4 rats with 3/4 thyroidectomies showed a definite degranulation of the acidophiles. In one case the granulation seemed to be normal, and in the other increased. In 2 animals some increase in the number of basophiles was noted.

Thyroid. Seven days after subtotal thyroidectomy in stalk-cut animals the residual

thyroid tissue showed a mean increase of 33.5% in the CHI (table 2). Although the cell height determinations were made from different lobes of the thyroid, that is, from the left at the time of operation and from the right lobe 7 days later, the figures are comparable. It has been shown previously that in normal rats the CHI of the 2 lobes do not significantly differ from each other (1).

Subtotal thyroidectomy after stalk section failed to produce any significant changes noticeable after 7 days in the adrenals, testicles, or seminal vesicles.

DISCUSSION

The regulation of thyrotropic function. The cytological changes noted in the anterior hypophysis of animals receiving thyroxin were similar whether the pituitary stalk was intact, completely severed, or only partially cut. These changes involved some increase in the number of acidophiles and basophiles, and an augmented granulation in the acidophiles. Subtotal thyroidectomy led to equivocal cytological changes, with a tendency toward degranulation of the acidophiles and an increase in the basophiles. These results are in agreement with the findings of other authors in intact animals (4). Evidently an increase or a decrease in the circulating thyroxin level produces cytological changes in the anterior pituitary independently of the pituitary stalk.

The thyrotropic function of the anterior pituitary is influenced by fluctuations in thyroxin level regardless of whether the pituitary stalk is intact or has been cut. This is evidenced by the observation that 10 days of thyroxin treatment, whether the stalk be intact or severed, causes a thyroid atrophy equal to that seen 10 days after total hypophysectomy (1). Moreover, subtotal thyroidectomy seems to stimulate the thyrotropic function sufficiently to give a marked increase in the CHI of the residual thyroid tissue even in animals with a completely severed pituitary stalk.

Since the pituitary stalk has been ruled out by these experiments, only the cervical sympathetics and direct humoral pathways are left to be considered as possible mediators of these thyroxin effects on thyrotropic function. That the cervical sympathetics play no important rôle in this relationship is suggested by certain earlier experiments showing that although cervical sympathectomy decreases temporarily and slightly the thyrotropic function, there is no continuing or permanent effect on the thyroid (1). This indicates that the thyrotropin-thyroid relationship continues normally in the absence of cervical sympathetics. Another experiment described in an earlier paper (1) points to the same conclusion. It was observed that prolonged exposure of an animal to cold causes increased sympathetic activity and stimulates the need for augmented secretion of the thyroid, resulting in thyroid hypertrophy. Initially cervical sympathectomy decreases slightly the stimulating effect of cold on the thyrotropic function of the pituitary. Later, however, the thyroid-thyrotropin relationship returns to normal in spite of cervical sympathectomy.

In conclusion our previous and present experiments indicate that the regulation of thyrotropic function depends primarily on humoral pathways with variation in the organism's thyroxin level as the most important factor (basic thyrotropic secretory rhythm). It seems apparent that this basic secretory rhythm continues to be normal under ordinary environmental conditions even after the destruction of the hypophyseal stalk (2, 3). However, as has been demonstrated previously, under certain circumstances such as exposure to cold, stimuli from the hypothalamus are transmitted through the pituitary stalk modifying the basal secretory rhythm of thyrotropin (2, 3). The fact that the data in the present paper are similar, both quali-

tatively and quantitatively, in intact animals and after stalk section, indicates that fluctuations in the blood thyroxin level do not belong to the modifying factors which act through the hypothalamo-hypophyseal pathways.

The cervical sympathetics can modify the thyrotropic function temporarily and slightly, although they are not necessary for a continued normal thyroid function (1). Apparently the modifying effect of the cervical sympathetics is less important than that of either the humoral or the hypothalamo-hypophyseal pathways (3). It is not yet clear whether the slight modifying effect of cervical sympathetics is due to a direct sympathetic secretory innervation of the hypophysis or to effects secondary to vasomotor changes.

Our concept of the probable mechanisms involved in the control of the thyrotropic function of the pituitary is schematically represented in the accompanying diagram.

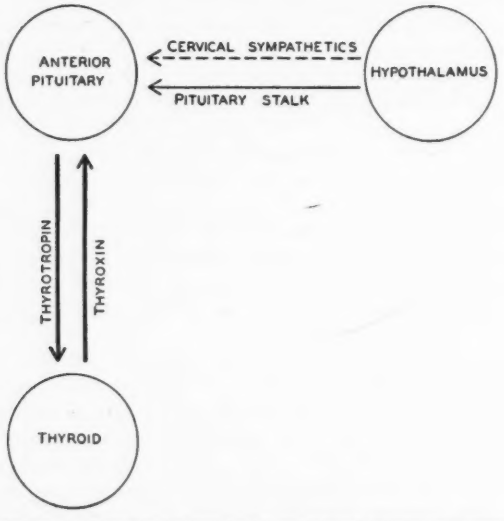

DIAGRAM DEMONSTRATING THAT THE THYROTROPIC FUNCTION IS PRINCIPALLY REGULATED BY THYROXIN THROUGH DIRECT HUMORAL ACTION (BASAL THYROTROPIC SECRETORY RHYTHM). This basal relationship can be modified by hypothalamo-hypophyseal pathways (cold reaction) and to a lesser extent by cervical sympathetics.

The effect of thyroxin on the adrenals and gonads. Thyroxin treatment causes adrenal hypertrophy whether the pituitary stalk be intact or severed. Since the adrenals also hypertrophy after cold or estrin treatment, even when the stalk is cut (3, 18), one must conclude that the corticotropic function is virtually independent of the pituitary stalk.

The present experiments show that after stalk section the appearance of the testes and the atrophy of the seminal vesicles after thyroxin injection do not appreciably differ from that produced by thyroxin in intact animals.

The mechanism whereby thyroxin produces atrophy of the accessories is not clear, though Smelser (19) states, on the basis of his experiments, that it depends in part on a decreased sensitivity of the seminal vesicles to male hormone and in part on a decreased effectiveness of the gonadotropins. However, before it can be established that thyroxin does not actively depress the gonadotropic function of the hypophysis,

actual determinations of the LH content of the anterior pituitary must be made. Since both luteinizing hormone and thyrotropin have been suspected of being produced by the same cells (acidophiles) (4), it would not be surprising if thyroxin affected directly both of these functions.

SUMMARY

An increase or decrease in an organism's thyroxin level acts on the anterior pituitary without any mediation or modifying action of the pituitary stalk. This is evidenced by the following findings.

Thyroxin given to male rats in daily 5 mg./kg. doses for 10 days causes a marked thyroid atrophy whether the pituitary stalk be severed or intact. Furthermore, other effects produced by thyroxin injection, such as weight loss, cytological changes in the anterior pituitary, adrenal cortical hyperplasia and atrophy of the seminal vesicles, are similar in stalk-cut or intact animals.

Residual thyroid tissue is still capable of compensatory hyperplasia after subtotal thyroidectomy even after the pituitary stalk has been destroyed.

It is concluded that the thyrotropic function of the anterior hypophysis has a basic secretory rhythm which is for the most part controlled humorally by variations in the organism's thyroxin level without the mediation of the hypothalamo-hypophyseal pathways. Hypothalamic impulses through the pituitary stalk, or to a lesser extent the cervical sympathetics, can under certain conditions modify this basic thyrotropic secretory rhythm.

The author wishes to express his gratitude to Dr. George B. Wislocki for his interest and criticisms during the work, and to Dr. H. Stanley Bennett who corrected the English.

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THE EFFECT OF THYROTROPIC HORMONE UPON PREGNANT RABBITS

BEATRICE WHITESIDE

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THE FACT THAT a relationship exists between the thyroid gland and the course of pregnancy has long been known. The normal reaction of the thyroid to pregnancy is a hyperplasia. On the other hand the injection of thyroxin into pregnant animals causes abortion or death and degeneration of the fetuses. In the last few years a number of experiments with the thyrotropic hormone have been carried out which prove that under the influence of the hormone the thyroid enters upon a state of hyperactivity. There remained the question as to whether or not the administration of the thyrotropic hormone influences gestation. Glaubach (1) published the results of an investigation along these lines. She proved that the injection of thyrotropic hormone into pregnant guinea pigs and rabbits disturbed the pregnancy and caused either an abortion or the death and degeneration of the fetuses.[1]

Glaubach (1) injected thyrotropic hormone into pregnant guinea pigs and rabbits. In many cases an abortion occurred, particularly if the treatment was started near the end of pregnancy. If the injections were begun about the middle of gestation and continued over a long period and the animal then killed the fetuses were found to be small, undeveloped and macerated. Similar results were obtained by Grumbrecht and Loeser (2) working on guinea pigs and rats. On the other hand, Doederlein (3) stated that 3 guinea pigs which were given large amounts of thyrotropic hormone gave birth to living young. On the whole, however, the administration of thyrotropic hormone to pregnant animals causes an interruption of the gestation. This result is similar to that obtained by injecting thyroxin (4–7).

Histological studies of the thyroid of nonpregnant animals treated with thyrotropic hormone have proved that hypertrophy and hyperplasia result (8–11). It is interesting that after long continued treatment with thyrotropic hormone the thyroid becomes refractory and involutional changes appear (12, 13, 14). Many theories have been advanced to account for this fact (15–18).

Investigations of the thyroid of pregnant animals after treatment with thyrotropic hormone are complicated by the fact that during gestation there is a normal increase of the activity of the thyroid. Bernard (19) found in guinea pigs at the beginning of gestation an increased activity of the thyroid, which decreased about the 32nd day but returned about the 42nd day and lasted until a few days after parturition. Verdozzi (20) reported similar observations.

Doederlein (3) and Grumbrecht and Loeser (2), working on pregnant guinea pigs and rats that had been treated with thyrotropic hormone, report a definite hyperplasia of the

[1] The present paper is a continuation of this work. Dr. Susi Glaubach of the Pharmacological Institute of the University of Vienna turned some of her material over to Professor Patzelt, Director of the Histological-Embryological Institute of the University of Vienna, for histological investigation. At the suggestion of Professor Patzelt I undertook this work. It is a pleasure for me to thank Professor Patzelt for the hospitality of his laboratory and for his friendly help and advice. I am also indebted to Dr. Glaubach for her cooperation during the course of the work. The purpose of the present investigation is the histological study of the thyroid glands, the ovaries and the fetuses of pregnant rabbits that had been injected with thyrotropic hormone.

thyroid; Grumbrecht and Loeser state that the glands of the control animals were in a resting condition. The thyroid glands of the fetuses of treated animals were described by Doederlein as in a state of hyperplasia. He concluded that the thyrotropic hormone penetrates through the placenta. Grumbrecht and Loeser state that the thyroid of fetuses of normal animals also shows hyperactivity and therefore the passage of the hormone from the maternal into the fetal organism cannot be shown in this manner.

The ovary of pregnant rats and guinea pigs after treatment with thyrotropic hormone has been examined by Grumbrecht and Loeser (2). The ovaries were enlarged and contained corpora lutea of a reddish yellow color which appeared normal in every way. They do not describe any degenerative signs such as appear after treatment with thyroxin (4, 7). These investigators injected thyroxin into pregnant rats and found an increase in the number of follicles, many of which also showed signs of degeneration. Kunde (6) obtained similar results with rabbits.

MATERIAL AND METHOD

As stated in the footnote[1] the material was turned over to the writer by Dr. Glaubach. She had injected pregnant rabbits subcutaneously with thyrotropic hormone.[2] One milligram of this extract contained 10 guinea pig units. The experimental animals can be divided into 2 groups. The first group consisted of 6 rabbits that received daily over a period of 14 to 17 days an injection of 30 guinea pig units of thyrotropic hormone. The injections were started in the second week of gestation and the animals were killed on the 28th day of pregnancy. Four untreated pregnant rabbits were killed on the 28th day of gestation and served as controls. The second group was made up of 3 rabbits that received daily for a period of 5 days an injection of 30 guinea pig units of thyrotropic hormone. The injections were started on the 9th day of gestation and the animals were killed on the 14th day of pregnancy. As a control 3 untreated pregnant rabbits were killed on the 14th day of gestation.

The fetuses and the thyroid glands and ovaries of the maternal animals were fixed in formalin, embedded in celloidin and the sections stained with hematoxylin and eosin.

RESULTS

Fetuses. The injection of thyrotropic hormone into pregnant rabbits brought about an interruption of gestation. In every case there was either an abortion or death of the fetuses. In the first series of 6 rabbits that were injected for a period of 14 to 17 days and killed after 4 weeks of gestation there were 2 cases of abortion and 4 cases in which dead and degenerated fetuses were found in the uterus. The fetuses were about the size of a bean (3 cm.). Sometimes the fetuses could be recognized outwardly as such but more often they were unformed masses. In the second group of 3 rabbits injected for 5 days there were 2 abortions. In the uterus of the third rabbit there were dead and slightly macerated fetuses. Microscopical investigation of the fetuses of the first group proved that in the majority of cases complete degeneration of the organs had taken place, and that the fetuses were partly resorbed. Much necrotic tissue was to be seen with here and there a patch of what appeared to be cartilage or epithelium. Large numbers of phagocytic cells were present. The placenta showed conspicuous signs of degeneration. In the few cases in which the fetuses were not degenerated to such a degree the organs seemed normal. The condition of the thyroid gland might be called slightly hyperplastic. The follicular epithelium was cuboidal and there was not very much colloid present. However the histological structure of the thyroid of untreated fetuses is very similar. These findings agree with those of Grumbrecht and Loeser (2) who worked on guinea pigs.

[2] The preparation was donated by the firm of Schering-Kalbaum.

In the second group of rabbits killed on the 14th day of gestation the histological appearance of the organs was normal. The thyroid was in an early stage of development.

Maternal animals, thyroid. The reaction of the thyroid of pregnant animals to the injection of thyrotropic hormone is complicated by the fact that the pregnancy itself produces hyperactivity of the thyroid. The question to be answered is therefore whether or not the administration of thyrotropic hormone causes a higher degree of hyperplasia than would be brought about by the pregnancy. In the investigation of this question the histological structure of the thyroid of untreated pregnant rabbits will be discussed first and then the appearance of the gland of injected animals.

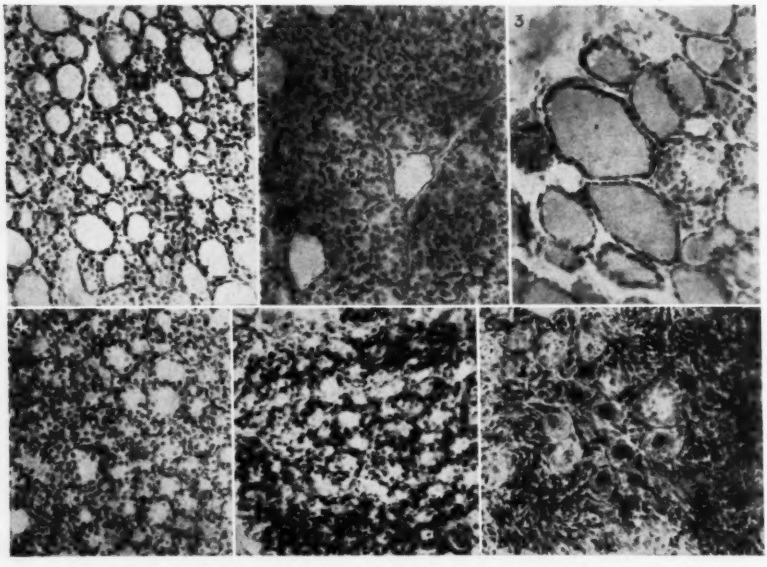

Fig. 1. Section of maternal thyroid gland of a control rabbit of group I showing the slight hyperplasia of a normal gland at end of pregnancy. Fig. 2. Maternal thyroid gland of injected rabbit of group I showing marked hyperplasia. Fig. 3. Maternal thyroid gland of injected rabbit of group I showing involution. Fig. 4. Maternal thyroid gland of control rabbit of group II showing normal hyperplasia of gland on 14th day of pregnancy. Fig. 5. Maternal thyroid gland of injected rabbit of group II showing a slightly greater degree of hyperplasia than the control. Fig. 6. Maternal ovary of injected rabbit of group I showing degenerating ova. All figures are photomicrographs reproduced at a magnitude of × 138.

The first group of rabbits consisted of 6 animals that were injected with thyrotropic hormone for a period of 14 to 17 days beginning in the second week of gestation. The animals were killed on the 28th day of gestation. Control animals were killed at the end of 4 weeks of pregnancy. Figure 1 shows the thyroid of a control rabbit. The follicles were middle sized or small. The cells were rather regular in form, generally cuboidal. The colloid was not very plentiful, did not stain very deeply and was often vacuolated. Many of the follicles contained no colloid. There was not a large amount of connective tissue present and blood vessels were not very conspicuous. Particularly with regard to the amount of colloid the gland differed from that of a normal nonpregnant rabbit as described by Hertz and Kranes (10). On the whole it may be said that there was a slight degree of hyperplasia.

The thyroid gland of rabbits of the first group showed in 2 cases a very pronounced hyperplasia (fig. 2). The follicles were small and very irregular in form. The cells were generally columnar. There was very little colloid present. The stroma showed a great increase in extent and it was rich in cells and blood vessels. Mononuclear cell infiltration was to be seen. There were also many neutrophiles in the blood vessels. There appeared to be solid growths of cells and the gland made a very compact impression.

In 3 animals of the first series the hyperplasia was not so pronounced as in the above described case, but still greater than in an untreated pregnant rabbit. The follicles were irregular in form and separated by large amount of stroma. Numerous blood vessels were present, containing many neutrophiles. In two cases there was very little colloid to be seen. In one gland however there was a fairly large amount of colloid present. This fact might be interpreted as the beginning of an involution.

In one rabbit of the first group the thyroid presented a typical picture of the end stage of involutional changes (fig. 3). The follicles were extremely large. The lumen of a follicle was often 7 or 8 times as large as in the thyroid of a normal pregnant rabbit (compare fig. 1. Both photographs have the same magnification). The follicle were distended with colloid which stains deeply. The follicle walls were composed of flat epithelium resembling the lining of blood vessels. There was very little stroma present and the vascularity was reduced. The histological structure of this gland was similar to the type of involution after long continued treatment with thyrotropic hormone in nonpregnant animals. It is, however, to be noted that in the 6 pregnant rabbits, injected for a period of 14 to 17 days, only one pronounced case of involution was found. One other rabbit showed signs of a beginning regression of the hyperplasia. In the case of nonpregnant animals the incidence of involution was very high. The small percentage of cases of involution of pregnant rabbits as compared to nonpregnant ones was probably due to the condition of the glandular system in gestation.

The second group of rabbits was injected daily for 5 days and killed on the 14th day of gestation. The thyroid of a control rabbit showed a definite hyperplasia (fig. 4). There were many small follicles. Less than half of the follicles contained colloid. The follicle cells were cuboidal. There was a fairly large amount of connective tissue and the gland appeared compact.

In the injected rabbits of the second series (fig. 5) we found that the thyroid showed a greater degree of hyperplasia than the control. The follicles were smaller and more irregular in shape and there was still less colloid present. Often the colloid appeared fluid. The stroma was very cellular. In all 3 of the injected animals the histological picture was identical.

Ovary. In the ovaries of the rabbits of the first group that were injected daily for a period of 14 to 17 days there were histological changes that resembled those obtained by treatment with thyroxin. The degenerative process was evident in the primary follicles (fig. 6). In many cases the ovum appeared very abnormal. The nucleus was irregular in shape and there were scattered clumps of chromatin. Often the entire ovum seemed to be disintegrating. There was an apparent increase in the number of graafian follicles many of which however were degenerating. The ovum was often filled with nuclear débris. The membrana granulosa also showed degenerative signs. Many of the cells showed pyknosis and karyorrhexis of the nucleus. Often cells became detached from each other. The liquor folliculi appeared somewhat granular, as it contained small spherules probably of nuclear substance. The corpora lutea appeared normal but smaller than in the ovary of an untreated animal. There were many corpora albicantia present. These changes were found in the majority of

the rabbits that were injected daily for about two weeks. In the second series of animals that were treated for 5 days the signs of degeneration were not observed. These findings differ from the result obtained by Grumbrecht and Loeser (2). These investigators observed no degenerative changes in the case of guinea pigs and rats.

DISCUSSION

The results reported in this paper confirm the earlier work of Glaubach (1) and show that thyrotropic hormone administered to pregnant rabbits interrupts the gestation. This is in accord with the findings of Doederlein (3) and Grumbrecht and Loeser (2) who worked on rats and guinea pigs.

The question that naturally arises in connection with these results is whether the thyrotropic hormone works through the thyroid gland or whether it acts directly upon the fetus. The latter alternative is possible because Doederlein (3) has reported that the placenta is permeable for the hormone. This investigator found the thyroid of fetuses of injected guinea pigs in a state of hyperplasia in contrast to the gland of a normal fetus. There is, however, a difference of opinion in this respect, as Grumbrecht and Loeser (2) state that the thyroid of both treated and normal fetuses showed signs of hyperactivity.

The results reported in the present paper would support the findings of Grumbrecht and Loeser and their conclusion that the condition of the fetal thyroid cannot be taken as proof that the thyrotropic hormone penetrates into the fetus. Furthermore Aron (8) and Schittenhelm and Eisler (21) deny that the placenta is permeable for thyrotropic hormone.

The histological results of the present investigation could be taken as support for the theory that the action of the thyrotropic hormone in disturbing gestation is connected with the thyroid gland. In the rabbit there is a greater hyperplasia of the maternal thyroid after treatment with thyrotropic hormone than is present in normal pregnancy. Another indication that excess thyroxin is produced is that the ovaries show signs of degeneration similar to those found when thyroxin is administered. Grumbrecht and Loeser (2) have shown that the placenta is permeable for thyroxin. It would seem therefore that the most probable mode of action of the thyrotropic hormone is to cause the thyroid gland of the maternal animal to secrete an excess amount of thyroxin which penetrates through the placenta and by its toxic action kills the fetus.

SUMMARY

Pregnant rabbits were injected with thyrotropic hormone. In every case the gestation was interrupted, either by abortion or by the death of the fetuses. This result confirms the work of Glaubach (1).

A histological investigation was made of the fetuses and of the thyroid and ovary of the maternal animals. In the majority of the fetuses of rabbits that had been treated for 2 weeks the organs were extremely degenerated. In the few cases where the organs could be studied they seemed normal. In the fetuses of animals that had been treated for 5 days the organs were normal. The thyroid of the maternal animal was in a state of hyperplasia that was more marked than the normal hyperactivity of pregnancy. There was a small percentage of involution of the thyroid after long continued administration of the thyrotropic hormone. The ovary of the maternal animal treated for 2 weeks showed evidences of degenerative changes similar to those caused by administration of thyroxin. In the case of rabbits injected for 5 days the ovary appeared normal.

The histological results presented in this paper would seem to indicate that the

thyrotropic hormone causes interruption of gestation by stimulation of the thyroid gland.

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AUTONOMIC CONTROL OF THYROID SECRETION

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THE AUTONOMIC control of thyroid function has been the subject of an extensive controversial literature. Investigation of this problem in numerous ingenious histological, physiological and chemical experiments has not resulted in its solution. Nevertheless, the impression persists that the autonomic nervous system is somehow related to regulation of thyroid function.

Advances in knowledge of the physiology of the thyroid gland were practically at a standstill just prior to the discovery of the anterior hypophyseal thyrotropic hormone. That discovery, aside from its significance in demonstrating an important relation between the anterior pituitary and thyroid glands, gave fresh impetus to the investigation of various phases of thyroid function by offering a new experimental approach. For this reason we returned to the study of a problem which had been the subject of a series of experiments in this laboratory about twenty years ago. Our present efforts were planned from several points of view.

We repeated the experiments of Cannon, Binger and Fitz (1) and Cannon and Fitz (2), who found that the cardinal signs of hyperthyroidism developed in a number of cats as a result of a unilateral phrenico-sympathetic anastomosis.

We conducted studies to determine whether or not the anterior hypophyseal thyrotropic hormone was physiologically effective in the absence of the cervical sympathetic chain and ganglia and the splanchnic innervation of the adrenal glands.

We planned to ascertain the effect on the metabolic rate of the prolonged administration of ephedrine, adrenaline, mecholyl and pilocarpine, which stimulate various parts of the autonomic nervous system. The details of these experiments were modified after the publication of a report by Uhlenhuth, Van Slyke and Mech (3), who found that the rapidity of metamorphosis of salamanders by a thyrotropic hormone could be significantly enhanced by the simultaneous administration of pilocarpine or adrenaline. We arranged, therefore, to study the rabbit and the guinea pig under similar experimental conditions. Miss Bevin and Dr. Uotila subsequently became collaborators in this phase of our work (4).

Phrenico-sympathetic anastomosis. During 1935–36 end-to-end anastomoses were made in 28 cats between the proximal portion of the right phrenic nerve and the distal segment of the cervical sympathetic trunk. The union was effected with various lengths of the whole phrenic or with one of its roots. The animals were observed at least 6 months before any anastomoses were tested physiologically with a faradic current. Twelve of the 28 cats in whom functional union of the nerves appeared to have taken place were selected for this test. In 9 of the 12 the anastomoses were found to be functional, as indicated by dilation of the pupil and retraction of the nictitating membrane in response to stimulation of the phrenic proximal to the anastomosis. Only 2 cats in the entire series (*Grey Lady* and *Tiger Lady*) developed the characteristic signs which were described by Cannon and his collaborators, viz., an increased basal

[1] Fellow of the National Research Council.

metabolic rate, tachycardia and restlessness. *Gray Lady* also had unilateral exophthalmos and respiratory hippus. There was no evidence of any change in the other cats in whom the nerves were proved to be physiologically united.

The nerves were anastomosed in *Grey Lady* on February 7, 1935. Not until 9½ months later, on November 22, 1935, was our special attention directed to her, although she had been observed periodically at more or less regular intervals until that time. She then had a right unilateral exophthalmos and tachycardia (fig. 1). The right pupil, larger than the left, showed respiratory hippus which could be seen best in a darkened room. During the preceding month it had been noted that she ate avidly, gulping down quickly her allotted food. Studies of her basal metabolism revealed a substantial increase in the rate of oxygen consumption as compared with the preoperative level (table 1 and fig. 2).

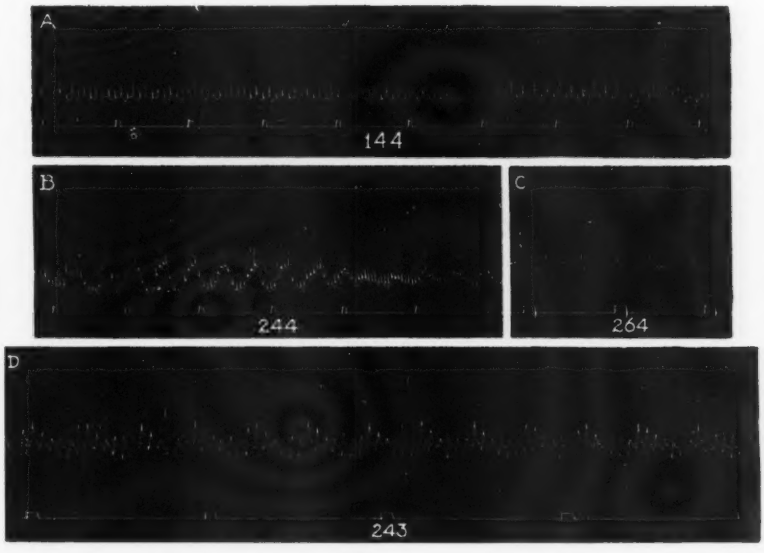

Fig. 1. KYMOGRAPHIC RECORD OF HEART BEATS WITH ANIMAL QUIET. Time intervals, 5 sec. *A*, Normal cat, 144 beats per minute. *B*, *Grey Lady*, 244 beats per minute on 11-23-35. *C*, *Grey Lady*, 264 beats per minute on 11-23-35. *D*, *Tiger Lady*, 243 beats per minute on 11-25-35.

After completion of her metabolism tests by the closed-circuit method on the morning of November 25, Dr. Gordon Ring made a series of similar observations during the afternoon by the open-circuit method with a Haldane apparatus. His results, like ours, indicated that the metabolic rate was significantly elevated as compared with her normal level (cf. table 1).

Closed-circuit method		*Open-circuit method*	
B. M. R.		B. M. R.	
Cal./sq. m./hr.	Cal./kg./hr.	Cal./sq.m./hr.	Cal./kg./hr.
44.5	2.99	40.4	2.89

On November 26, 1935, *Grey Lady* died suddenly without warning, during her fourth metabolism test. There was no immediate explanation for her death, nor was

anything discovered at autopsy which accounted for it. She did not appear ill before the test was started; and she had been eating voraciously the day before her death. It may be of importance that just before she died during the early minutes of the final test, she was greatly excited and twisted and turned about in the metabolism chamber in an agitated manner. Her B.M.R. was elevated markedly during the inter-

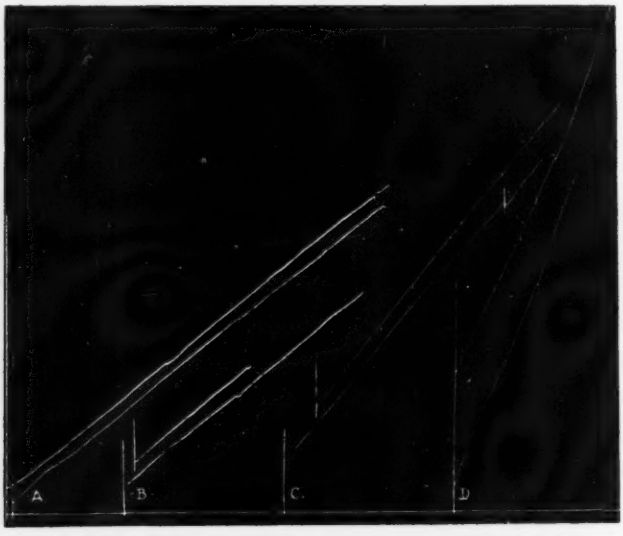

Fig. 2. KYMOGRAPHIC RECORD OF SLOPE OF B.M.R. CURVES. Gross muscular movement can be detected by a break in the linear continuity of the line, e.g., the lower of the two curves in C. *A, Cat 15* normal animal. B.M.R. equals 26 Cal. per sq. m. per hr. *B, Grey Lady.* B.M.R. on 2-1-35 equals 27.81 Cal. per sq. m. per hr. Average B.M.R. before operation 29.36 Cal. per sq. m. per hr. *C, Grey Lady.* B.M.R. 9½ months later on 11-23-35 equals 44.1 Cal. per sq. m. per hr. *D, Grey Lady.* B.M.R. on 11-26-35 not calculated. Curves made during test just prior to death.

mittent quiet periods (fig. 2, D). At autopsy, her thyroid glands did not show any significant change in cell height as compared with the normal average; however, the colloid was thin in consistency and scanty in amount, and the acini were more numerous and somewhat smaller than those usually seen in glands which have never been hyperplastic. Similarly, the interfollicular cellular tissue seemed hyperplastic.

TABLE 1. A CHRONOLOGICAL RECORD OF EXPERIMENTS WITH *Grey Lady*

Date	B.M.R. Cal./sq.m./hr.	Weight
		kg.
2- 1-35	27.81	3.517
2- 6-35	31.98	3.500
2- 7-35	Right phrenico-sympathetic anastomosis	
2- 8-35	28.29	3.568
11-22-35	Right eye protrudes more than left Right pupil is greater than left Respiratory hippus, right pupil (in dark) Eats voraciously	
11-23-35	44.1 (+47.5%)	3.165
11-25-35	44.5 (+49%)	3.200

TABLE 2. A CHRONOLOGICAL RECORD OF EXPERIMENTS WITH *Tiger Lady*. RIGHT PHRENICO-SYMPATHETIC ANASTOMOSIS—MAY 14, 1936

Date	B.M.R. Cal./sq.m./hr.	Weight	Pulse rate per min.
		kg.	
11-17-36	36.40	2.52	
11-18	36.80	2.55	
11-19	37.10	2.57	264
			268
11-21	35.39	2.67	288
11-23	34.13	2.65	
11-24	Right thyroidectomy. Carotid pulsations and heart rate taken under deep ether anesthesia were 228–240 per min. A normal control animal observed in similar circumstances at the same time had a pulse rate of 180 per minute.		
11-25	37.83	2.58	240
12-14	33.03	2.57	
12-17	35.68	2.50	
12-18	33.14	2.65	
12-23	31.85	2.52	
12-30	31.96	2.78	
1- 7-37	32.48	2.59	
1- 4			226
1-18			184
1-20	31.85 (more placid than formerly)	2.65	168
1-25	28.50	2.77	
1-28	28.94	2.82	180
2- 2	29.88	2.92	180
2- 5	31.51	2.91	
2-11	32.63	2.98	192
2-16	31.49	3.03	
2-24	30.18	3.11	
3- 4	31.16	3.27	
3- 5			200
3-15	Removed phrenico-sympathetic anastomosis. Carotid pulsations and heart rate taken under deep ether anesthesia were 216 per min.		
3-18	29.19	3.27	
3-22	26.38	3.15	
3-23	27.82	3.13	
3-29	26.56	3.10	216
4-12	31.16	3.67	
5-12	30.18 (easy to manage, less restless)	3.66	216
5-22	29.17	3.71	216
6- 3	30.64	3.67	
6-11	31.00	3.76	204

Tiger Lady, the second cat, was subjected to a right phrenico-sympathetic anastomosis on April 14, 1936. Determinations of her metabolic rates were not made at this time. Seven months later she was noticeably restless and difficult to handle. On November 17 her basal metabolism was found markedly elevated in comparison with the average rate for normal cats, which in our observations was approximately 26 calories per square meter per hour. *Tiger Lady* also had a concomitant tachycardia (table 2, fig. 1). Stimulation of the proximal segment of the phrenico-sympathetic anastomosis caused retraction of the nictitating membrane and pupillary dilation at the time of the right thyroidectomy, on November 24, 1936. The heart rate was not accelerated by the faradic stimulation. The histological appearance of the removed lobe was identical with that described for the thyroid in *Grey Lady*. Eight weeks after removal of the thyroid lobe on the side of the anastomosis the basal metabolism

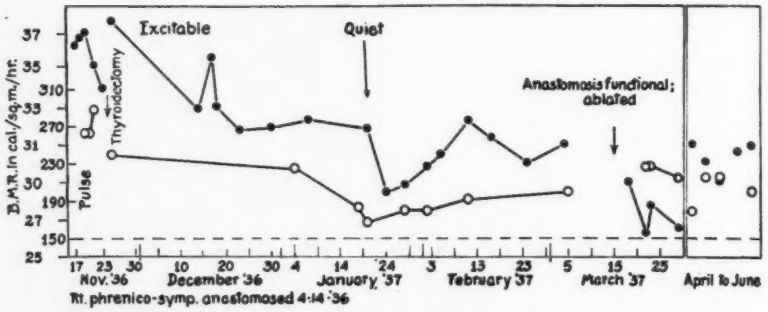

Fig. 3. EXPERIMENTAL COURSE OF *Tiger Lady*.

of *Tiger Lady* reached its lowest level for the condition. It dropped even lower within 10 days after excision of the anastomosis, which was found to be functional by electrical stimulation immediately prior to its removal (table 2, fig. 3).

Anterior hypophyseal thyrotropic activity after cervical sympathectomy. In 1934, 4 docile cats were selected for these experiments after a number of preliminary metabolism determinations had been made in order to prepare them adequately for such studies. Two, as controls, were injected intraperitoneally once daily with 5 cc. of an anterior pituitary extract (Squibb) which was known to contain the thyrotropic fac-

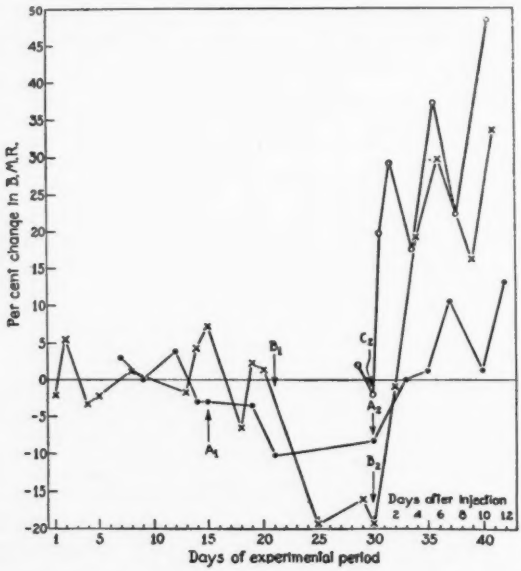

Fig. 4. A STUDY OF THE EFFECT OF VARIOUS OPERATIONS ON THE ACTIVITY OF THE ANTERIOR HYPOPHYSEAL THYROTROPIC HORMONE. A_1, Bilateral cervical sympathectomy. B_1, Bilateral superior cervical ganglionectomy, denervation of right adrenal, removal of left adrenal. A_2, B_2 and C_2, Points at which daily injections of the thyrotropic hormone were begun in doses of 5 cc. each.

tor in significant quantities (5, 6). Both cats responded to the thyroid-stimulating effects of the extract with a maximal increase in basal metabolism of 35 and 48%, respectively, in 10 days (fig. 4, *cat C*). The other two cats were operated upon. One had both cervical sympathetic chains dissected out and evulsed (*cat A*); the other was subjected to bilateral superior cervical sympathetic ganglionectomy, left adrenalectomy and denervation of the right adrenal (*cat B*). During the 5 days following these operations the basal metabolism of both animals decreased significantly (fig. 4, *cats A* and *B*). Injections of the thyrotropic hormone were begun after the animals had made a complete postoperative recovery and the basal rate of metabolism had been stabilized at its new level. As a result of this treatment the rate of metabolism became accelerated in both animals, but the increase was more marked in *cat B*. Similar individual differences in response are well recognized in the guinea pig (6); it is probable, therefore, that the difference noted here is of no importance. It was concluded that neither cervical sympathectomy nor denervation of the adrenals interfered with the characteristic metabolic effects of the thyrotropic hormone; and note was made of the marked decrease in metabolism (−20%) which followed the operations in *cat B*.

Augmentation of anterior hypophyseal thyrotropic hormone activity with pilocarpine and adrenaline. A preliminary group of 3 rabbits were injected daily with a solution of thyrotropic hormone (ox pituitary) prepared and generously given to us by Dr. J. B. Collip. The first rabbit received only the thyrotropic hormone; the second rabbit was given this hormone plus adrenaline; and the third rabbit was injected with this hormone plus pilocarpine. The first rabbit was not affected appreciably within 11 days by the thyrotropic activity of the extract in the doses given. The B.M.R.'s of the other 2 rabbits, which were injected with adrenaline or pilocarpine in addition to the thyrotropic hormone were increased approximately 27% within 9 and 11 days, respectively.

The experiments were now continued with guinea pigs to avoid giving the large amounts of thyrotropic hormone necessary to activate the rabbit thyroid. Before proceeding with this program an initial test was made of the effects of daily injections of adrenaline, pilocarpine and mecholyl on the basal metabolism of guinea pigs. The injections were given 3 times a day at equal intervals between the hours of 9 A.M. and 5 P.M., but on the day of the metabolism examination they were withheld until the test was finished in order to avoid possible immediate metabolic effects of the injections. All injections were discontinued on the 8th day of the experiment, the last treatment having been given on the 7th day at 5 P.M.

The results of these experiments indicate that neither adrenaline, pilocarpine nor mecholyl increases the B.M.R. of guinea pigs during the days of administration. Instead, during the injection period the basal metabolism of most of the animals was on the minus side of the individual average normal rate. It is interesting to note that within 24 to 72 hours after the injections were discontinued the B.M.R. had decreased remarkably and continued low for several days in all instances. The significance of this phenomenon is not apparent at present, but merits further investigation. The fact that the most marked decreases in metabolism occurred after the injections were discontinued suggests that the injected substances were responsible for a metabolic effect, possibly masked, to which a reaction later took place in the opposite direction.

With the assurance that adrenaline and pilocarpine did not increase the metabolic rate, we proceeded with the investigation. One group of guinea pigs was injected

intraperitoneally once daily with 0.5 cc. or 1.0 cc. of the thyrotropic hormone. A second group was given identical injections plus 0.75 cc. of 1:100,000 adrenaline, subcutaneously, 3 times daily; and a third group was given pilocarpine hydrochloride, 0.16 cc. of an 0.6% solution, subcutaneously, 3 times daily in addition to the same amount of thyrotropic hormone. Although the animals in the second and third groups responded to the thyrotropic hormone more actively during the first 4 days than those in the first group, the difference was not sufficiently marked to warrant more than passing comment. It then occurred to us that perhaps the amount of thyrotropic hormone which was administered stimulated the thyroid so extremely that further increase of its effect was impossible. The daily dose of thyrotropic hormone was therefore decreased to 0.25 cc., but the same dosage of pilocarpine and adrenaline was continued. Two sets of the same experiment were run at different seasons of the

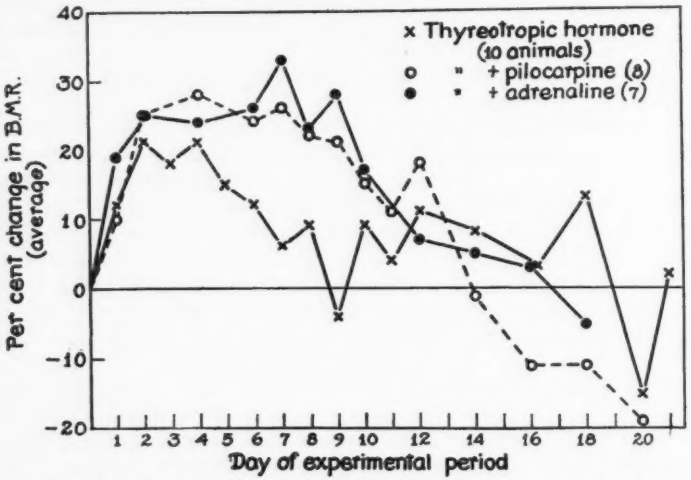

Fig. 5. Composite curves showing the average percentage change in B.M.R. in 10 guinea pigs receiving daily injections of thyrotropic hormone alone, 7 guinea pigs receiving daily injections of thyrotropic hormone and adrenaline, and 8 guinea pigs receiving daily injections of thyrotropic hormone and pilocarpine.

year. The one done in the spring resulted in more striking differences between the control and experimental groups than those completed during the winter, but the latter likewise showed that the joint administration of adrenaline or pilocarpine with the thyrotropic hormone (as contrasted with injections of the hormone alone) results in a greater immediate increase in metabolic rate as well as in a definite prolongation of the effect of the thyrotropic hormone. The results of these experiments have been summarized in figure 5.

DISCUSSION

The relation of the sympathetic nervous system to thyroid function is by no means clear, but there are certain facts in connection with this problem which are fairly well established.

The function of the thyroid gland proceeds without appreciable deviation from the normal and responds characteristically to physiological stimulation in the absence of the cervical sympathetic nerves. Eitel, Krebs and Loeser (7) and Friedgood (8) have found that the anterior hypophyseal thyrotropic hormone induces hypertrophy and hyperplasia of thyroid tissue in vitro in blood serum, and Marine and Rosen

have reported (9) that the hormone causes characteristic histological changes in autotransplanted bits of thyroid tissue. Our observations, likewise, show that the thyrotropic hormone stimulates the sympathectomized, but otherwise intact thyroid (*cats A* and *B*, fig. 4).

The weight of recent evidence is against the view that stimulation of the nerves to the thyroid gland is transmitted directly to the thyroid cells. There are those, however, who, because of clinical impressions, believe that the nervous system can play an important rôle in the activation of the thyroid gland.

On the basis of observations on the dog's thyroid, Nonidez (10, 11) has concluded that the autonomic nerve supply to the gland is vascular, not parenchymal; and Friedgood and Bevin (12) could not find a consistent effect on the rate of metabolism of rabbits in acute experiments which consisted of a 1 to 3-hour period of cervical sympathetic stimulation.

On the basis of available experimental evidence one may speculate on the ways in which activity of the autonomic nervous system might influence thyroid function. The striking changes in function of the extremities which are concomitant with the vasomotor disturbances in the clinical syndrome of causalgia (13) suggest that a prolonged and serious disturbance of the vascular system of the thyroid might similarly affect the functional state of the thyroid cells. Another possibility is that the nervous system operates through a neurohypophyseal pathway. It is conceivable that neural stimuli transmitted to the hypophysis by way of the infundibular stalk and perhaps through the cervical sympathetics might increase the rate of elaboration and secretion of the thyrotropic hormone and could thus induce a state of hyperthyroidism indirectly. There is evidence that either one of these neural pathways can increase the gonadotropic activity of the anterior hypophysis. Brooks (14) and Westman and Jacobsohn (15) found that coitus initiates an impulse which passes through the infundibular stalk of the rabbit at one point in its course to the anterior pituitary gland and thus induces ovulation; others (16, 17) have produced ovulation in the rabbit by stimulation of various localized areas in the hypothalamus. That the cervical sympathetics influence the gonadotropic activity of the anterior pituitary to a limited extent seems likewise certain. Collin and Hennequin (18) have demonstrated that ablation or faradic stimulation of the cervical sympathetics induces significant changes in the cytologic appearance of the anterior pituitary gland. Friedgood and Pincus (19) and Friedgood and Cannon (20) have found that cervical sympathetic stimulation induces maturation of rabbit ova by increasing to a limited extent the gonadotropic activity of the anterior pituitary. It has been observed, furthermore, that ablation of the cervical sympathetics in the rat interferes significantly with the gonadotropic activation of the pituitary by glass-rod stimulation of the cervix uteri (21). In accord with these suggested pathways, Uotila (22) has claimed that exposure to cold stimulates the rat's pituitary by way of the infundibular stalk and thus activates the thyroid gland by means of the thyrotropic hormone. The effectiveness of such a neurohypophyseal mechanism could be increased by a hormone, such as adrenaline, since the latter is shown to augment the effect of the thyrotropic hormone on the thyroid gland (cf. fig. 5).

The significance of this augmentation phenomenon is as yet obscure. Our experiments with guinea pigs confirm in general the results obtained by Uhlenhuth, Van Slyke and Mech (3) in salamanders, although a clear difference between a greater augmenting effect of pilocarpine as compared with that of adrenaline was not found in accordance with their observations.

Since the modus operandi of this augmentation is important, it is pertinent to inquire into the known pharmacologic actions of pilocarpine and adrenaline. The

effects of adrenaline are for the most part identical with those of stimulation of the sympathetic nerves; pilocarpine, on the other hand, stimulates those parasympathetic peripheral structures which are paralyzed by atropine. The similarity of the augmenting effects of pilocarpine and adrenaline seems rather surprising in view of this difference in their pharmacologic behavior. Pilocarpine has effects, however, which offer a possible explanation for the apparent paradox. Dale and Laidlaw (23) and Houssay and Molinelli (24) demonstrated that pilocarpine increases the rate of secretion of adrenaline from the adrenal medulla. This suggests that the augmenting effects of pilocarpine may be exercised through the adrenal medulla. The evanescence of the effects of *injected* adrenaline may account for the more striking results which Uhlenhuth and his collaborators obtained with pilocarpine in augmenting the pharmacological activity of the thyrotropic hormone in salamanders, which are very sensitive test subjects. It may be of interest to note also that both pilocarpine and adrenaline induce small increases in metabolic rate, immediate in onset, but of short duration (25, 26, 27). The rapidity with which this increase in metabolism occurs and subsides indicates that it is not related to thyroid activity. According to Kelemen (26) the increase in basal metabolism due to pilocarpine is antagonized by atropine. In our opinion the small changes in metabolism which are involved require a more extensive series of experiments than his in order to substantiate this point adequately.

Lacking evidence to the contrary, we are assuming for the present that adrenaline is the substance which is ultimately responsible for augmenting the activity of the thyrotropic hormone. Although adrenaline can induce an immediate increase in metabolic rate, it is clear from the data of the control experiments that it does not have a persistent or delayed effect which can be interpreted in terms of thyroid stimulation. From a theoretical viewpoint, adrenaline may take part in this augmentation in one of several ways.

(*a*) It may increase the potential effect of the thyrotropic hormone by stimulating the thyroid cells by way of their sympathetic innervation. This is improbable because recent evidence indicates that the sympathetic innervates the blood vessels, not the thyroid cells.

(*b*) Adrenaline may accelerate the rate of oxygen consumption by reinforcing the effects of thyroglobulin peripherally in the tissue cells. We have found that in normal guinea pigs neither adrenaline nor pilocarpine increases the metabolism-stimulating effect of desiccated thyroid substance. These experiments eliminate this second possibility, although we should like to see them repeated with thyroidectomized guinea pigs which are more sensitive test subjects.

(*c*) Adrenaline may act directly on the thyroid cells without reference to the nervous system, but in cooperation with the thyrotropic hormone. Since adrenaline alone does not increase the rate of secretion of thyroglobulin, it is possible that it renders the cell more sensitive to the thyrotropic activity of the anterior hypophyseal extract. We are inclined to accept this theory as the basis of a working hypothesis until more is known concerning the nature of the augmentation phenomenon.

Animals with phrenico-sympathetic anastomosis. An adequate explanation is still lacking to account for the difficulty which we and others have encountered in reproducing the results originally reported by Cannon, Binger and Fitz (1). One must emphasize, however, that in these circumstances one positive result carries more weight than many negative ones. Since we lack the means of reproducing this syndrome at will, it appears premature to speculate concerning its significance in

relation to the pathogenesis of exophthalmic goiter. Many of its features are frankly baffling. To what physiological mechanism, for instance, can one attribute the increased rate of metabolism?

That the moderate depression of the metabolic rate which took place after a hemi-thyroidectomy was further accentuated by removal of the ipsilateral phrenico-sympathetic anastomosis (fig. 3; table 2) suggests two things, viz., first, that the anastomosed nerves were contributing to the increase in metabolic rate, and, second, that this neural stimulus was not acting directly upon the ipsilateral thyroid lobe. The latter is in accord with data previously discussed. Other than the thyroid gland, however, there is evidence that the cervical sympathetics innervate the anterior pituitary (which secretes a thyrotropic hormone), for previous studies indicate that sympathetic impulses affect this pituitary lobe so as to stimulate, though to a minor degree, its gonadotropic activity. Stimulation of the pituitary through a phrenico-sympathetic anastomosis in an unanesthetized animal over a period of months might excite its thyrotropic function. Although the histological appearance of the thyroid gland in this syndrome is somewhat suggestive of a previous hyperactive state, the characteristic findings of the latter were absent even when the metabolic rate was considerably elevated. This adds considerably to the difficulty of interpretation. Is it possible that the secretion of the anterior pituitary which is concerned is the metabolic hormone described by O'Donovan and Collip (28) and Billingsley, O'Donovan and Collip (29)?

Another unexplainable feature is the nature of the unilateral exophthalmos. Sappey (30), MacCallum and Cornell (31) and Code and Essex (32) have shown that in dogs stimulation of the cervical sympathetic fibers affects a contractile cuff of the circular fibers of the *musculus orbitalis* which thereupon squeeze the eyeball forward. The apparent significance of these findings has been tempered somewhat by more recent observations (33) which indicate that the dog's eyeball recedes 2 to 6 mm. during anesthesia, and that stimulation of the cervical sympathetic merely restores the globe to its pre-anesthetic position. Exophthalmos, however, does not occur under these experimental conditions. They also found that during anesthesia sympathomimetic drugs resulted in changes similar to those elicited by neural stimulation; but no alteration in the position of the eyeball occurred in the unanesthetized animal. It is as yet unknown how the eyeball would respond to electrical excitation of the cervical sympathetics in the unanesthetized dog. Aside from any neural effect there is an anterior pituitary hormone which in amphibia, birds and mammals induces exophthalmos. Since this substance must exert its effect through the circulation, one would expect that the position of both eyeballs would be affected if this exophthalmos-producing substance were implicated.

The tachycardia found in the two animals may be related to the concomitant increase in B.M.R. It is not due to a stimulatory effect upon the cardiac nerves by phrenic impulses. This conclusion is based on the observation that stimulation of the proximal segment (phrenic) of the anastomosis failed to accelerate the heart rate, although the pupil became dilated and the nictitating membrane retracted promptly.

The presence of markedly hypertrophied adrenals in one of Cannon and Fitz's original cats (2) recalls the characteristic effect of the adrenotropic hormone of the anterior pituitary and lends some credence to the view that the anterior lobe of the pituitary is stimulated in some way through the anastomosis.

SUMMARY

In 2 of 28 cats a right phrenico-sympathetic anastomosis resulted in a syndrome originally described by Cannon, Binger and Fitz (1) (fig. 1, 2, 3 and tables 1, 2). Removal of the ipsilateral thyroid lobe in one cat was followed by a moderate but progressive decrease in the basal rate of metabolism; and subsequent resection of the anastomosis brought the metabolic rate down to a normal level within 10 days after operation (fig. 3 and table 2).

The cervical sympathetic trunks and the splanchnic innervation of the adrenals are not essential to the action of the anterior hypophyseal thyrotropic hormone. These operations were followed by a significant decrease in the basal rate of metabolism in the 2 animals studied (fig. 4).

The daily injection into normal guinea pigs of adrenaline, pilocarpine or mecholyl does not significantly alter the B.M.R. over a period of 8 days. The immediate effect of these drugs on the metabolic rate was not studied. Pilocarpine or adrenaline augment the thyroid stimulating activity of an anterior hypophyseal thyrotropic hormone (fig. 5). Certain theoretical considerations suggest that pilocarpine acts through the adrenal glands in eliciting its augmenting effect.

The question of sympathetic nervous system control of thyroid function is discussed in detail.

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A METHOD OF ASSAYING PITUITARY WATER RETENTION PRINCIPLE

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BOYD AND WHYTE (1, 2) have recently presented evidence of the presence in pharmacopoeial (British), acetic acid extracts of the neurohypophysis of at least two principles which may have a delayed effect upon water balance. It was shown (1) that such extracts inhibit the loss of body water in frogs and also (2) stimulate the loss of injected salt over a period of several hours. The net effect of administering such pituitary extracts must therefore depend upon the state of water and salt balance at the time of administration and upon the relative amounts of the two principles in the extract administered. The extract employed in this work, except where otherwise noted, was surgical pituitrin (Parke, Davis & Co.) which will hereafter be designated simply as pituitrin.

These two principles appear to have been responsible in part for the confusing 'antidiuretic' and 'diuretic' responses to pituitrin obtained by various investigators in the past (3, 4). Because the reactions of Boyd and Whyte (1, 2) and of other investigators in this laboratory (5–8) extend over a period of several hours, they do not appear to correspond to the pressor alterations which pituitrin may cause in the renal blood supply. Such pressor changes are in general short lived and can account for only evanescent variation in urine volume, variations over a period of half an hour at the most (9). In the light of available evidence, it would seem that pituitrin may have an early and a delayed effect on water metabolism, the early effect being due to circulatory changes. Whether the delayed effect is renal or extra-renal or both is at present a moot question.

As stated, the delayed effect appears to have at least two phases and the present investigation was concerned with a method of assaying the water retention phase. The available methods of measuring this latter activity of pituitary extracts are based upon measurement of the volume of urine and have been reviewed by Nelson and Woods (10), the rat method of Burn (11) being perhaps the most extensively used. These methods measure changes in the rate of loss of water from the body through one channel only, the kidneys. There is evidence, for example that of Steggerda (12) when conjoined with that of Boyd and Whyte (1, 2), that pituitrin may alter the ability of extra-renal tissues to hold water. In the latter event, pituitrin might possess a much greater effect on the total loss of water than upon renal loss alone especially in animals such as the frog in which the renal loss of water is relatively insignificant when compared with the extra-renal loss.

The method of assay devised in the present investigation was based upon the effect of pituitrin on the total water content of the body of leopard frogs (*Rana pipiens*). It has been known for some time that pituitrin injected into frogs immersed in water increases the water content of the body (5). The usual interpretation of this reaction has been that pituitrin "alters the physiology of the skin" of frogs or more specifically stimulates the skin to absorb more water (4). Boyd and Whyte (1) presented data which indicated that the effect of pituitrin on amphibian water balance could be explained in a manner similar to that in higher animals and that no new mechanism need be invoked. In brief, they found that frogs in water are capable of taking up and losing considerable quantities of water hourly and that the amount of water which pituitrin "stimulates the skin to take up" is just that amount

which the body is normally capable of absorbing in the same interval, and losing if pituitrin is not injected. Hence they conclude that in frogs, as in higher animals, pituitrin simply causes a retention of water under the specified conditions of the experiment.

Boyd and Brown (5) had made use of this reaction in a preliminary assay of posterior pituitary extracts. Subsequent use of the preliminary method has not been found entirely satisfactory in this laboratory because of variability in results and because of lack of specificity in defining the unit. Also because the response of the body water of frogs to pituitrin

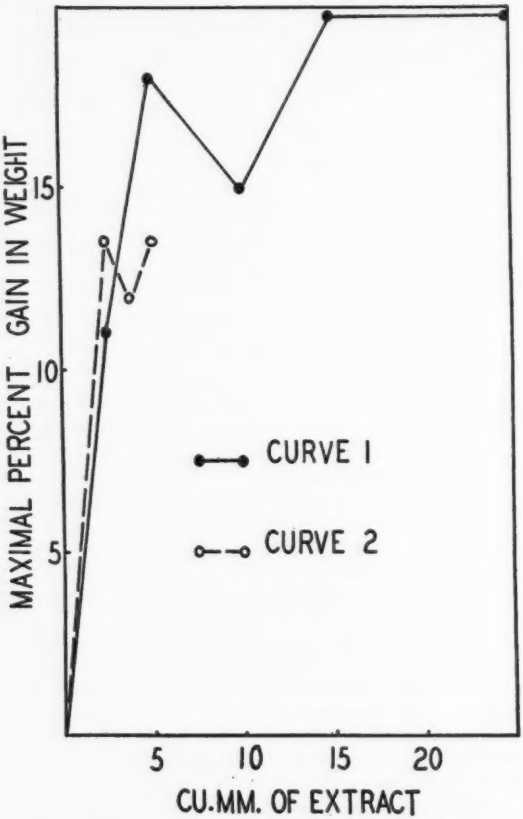

Fig. 1. An assay of pituitary water retention principle in frogs at room temperature.

varies with seasons, the original unit (5) has been subsequently found not feasible the year around. The present work was an attempt to improve the technic of assay and induce more flexibility and at the same time more specificity into the unit.

The Development of the Assay Method

The method of assay of Boyd and Brown (5), with certain important exceptions from the original method, was performed at room temperature in the following manner. Knowing the approximate minimal amount of extract which would yield maximal water uptake from preliminary trials, 30 frogs were assembled in 5 groups of 6 animals each. Each frog was then placed in 400 cc. beakers containing 100 cc. of

water and the beakers were covered with a weighted glass or board top to exclude air currents which were subsequently found to depress the uptake of water (8). The animals were then weighed to the nearest 0.1 gm. and each group injected into the dorsal lymph sac with a given dose of extract per 10 gm. of body weight and re-weighed at hourly or half-hourly intervals. The smallest amount of extract producing the maximal gain in weight was then considered as the frog unit of water retention principle.

The difficulties inherent in this procedure may be illustrated in a typical assay, in which the mean maximal increase in weight with each dose of extract per 10 gm. of body weight is plotted against the dose in cu. mm. (fig. 1). Curve 1 obtained on this particular extract suggested that the frog water retention unit was contained in 5 cu. mm. of the extract but a repeat curve, No. 2, was performed with 2.5, 3.75 and 5.0 cu. mm. and gave a confusing result. There was no significant statistical difference between any of the 3 points on curve 2 while the mean difference between the 2.5 and 5.0 cu. mm. doses of curve 1 was 8.2% with a standard deviation of 3.7 which was considered as statistically significant (13). Repeated assays on this same extract established the unit as contained *in the neighborhood* of 5 cu. mm. of extract but this required several days, making the method cumbersome, and the results were not clear-cut.

In these assays performed at room temperature, it became evident that different frogs reached a peak of water uptake at different times after injection. When frogs were weighed at hourly intervals the peak was frequently missed so that when the peak mean of a group was calculated it included some frogs which had passed the peak and some which had not yet reached the peak. It appeared that this difficulty might be overcome if the entire process could be slowed so that peaks would be held for a relatively long interval and that at a certain specified time in this interval all frogs would be at the peak; at the same time more animals could be included in the assay. Boyd and Brown (5) have shown that the entire reaction is slowed at low temperatures. The remainder of this investigation was then concerned with assays performed in a refrigerator room at a temperature of 4° C.

Acclimitization to 4° C. Frogs were found capable of holding more water at 4° C. than at room temperature. When frogs in water at room temperature are placed in a refrigerator room at 4° C. the normal weight of the frog increases and reaches a plateau value in 12 hours. In a group of 48 frogs so treated, the minimal increase in weight was 2%, the maximal 30% and the mean 12%. In the winter time when the temperature of running tap water (Lake Ontario) in the frog storage tanks went as low as 6° C., the frogs took up only 1 to 2% of water when placed in the refrigerator room at 4° C. This increase in body water was found maintained for a period of at least one week with very little fluctuation. For assay purposes, therefore, frogs must be kept in water at 4° C. for a period of at least 12 hours before being injected.

Response to pituitrin at 4° C. When doses of pituitrin sufficient to give a maximal water uptake are injected into frogs acclimatized to 4° C., there occurred a further increase in body water which began in from 1 to 5 hours and reached a maximum in from 9 to 16 hours. The maximum increase was then held for from 3 to 11 hours. Between 14 and 18 hours, 92% of the animals were holding a peak uptake and the remainder were within 1 or 2% of their peak. Subsequently the excess body water was gradually lost and in 48 to 96 hours (average 72 hours), all frogs were back to within 3% of their weight before injection. A typical result obtained in the summer months, in 18 animals is charted semi-diagrammatically in figure 2.

These results indicated that two weighings are sufficient for assay purposes, one

before injection and a second between 14 and 18 hours after injection of the extract. Thus was assured a correct measurement of the peak uptake of water and a saving of time in weighings which permitted more animals being used for the assay.

Subsequent use of frogs. The next question was do these frogs return to 'normal' after their experience in the refrigerator and if so what interval is required? It was found that frogs readily lose the additional water taken up at 4° C. when removed, still in water, to room temperature. The dotted lines in figure 2 indicate mean results of typical experiments in which it was found that within 3 to 6 hours after removal from a temperature of 4° C. all of the cold, with or without pituitrin, added water was lost.

To see if the water balance of such animals could now be considered normal,

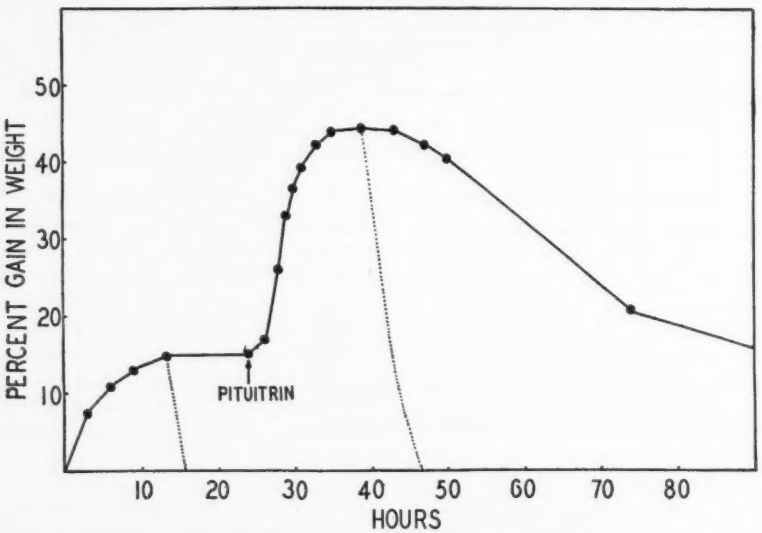

Fig. 2. THE UPTAKE OF WATER BY FROGS AT 4°C. AND INJECTED WITH PITUITRIN, SOLID LINE, AND CHANGES ON BEING BROUGHT TO ROOM TEMPERATURE, DOTTED LINE.

they were again injected with pituitrin and the resulting uptake of water at room temperature was identical to that before being placed in the refrigerator. As far as this reaction is concerned, the frogs appeared to have suffered no ill effects from their experience in the refrigerator room. Nor has the repeated use of frogs for these assay purposes appeared to affect their mortality rate compared with unused controls.

Peak interval and dosage. Markedly decreasing the dose of injected pituitrin lessened the time required to reach a peak of water retention and also lessened the duration of the peak plateau. None of these small doses were sufficient to produce a maximal uptake of water and doses within 25 to 50% of the smallest dose required to produce the maximal uptake of water (hereafter called the optimal water retention dose or O.W.R. dose) and all larger doses were at a peak response 18 hours after injection. Hence it was concluded that a second weighing made in 18 hours after injection would be satisfactory for assay purposes and a convenient interval to work with.

Number of animals. The next step investigated was the minimal number of animals required to give consistent mean results. As might be expected, the greatest

individual variations were encountered with doses which were 1/10th to 1/5th of the O.W.R. dose or less and the least variation with doses several times the O.W.R. dose. Five hundred and twenty frogs were then injected with a dose of pituitrin which was tentatively selected as being approximately the O.W.R. dose in order to study variations at this, the most important, dosage. It was found that amongst any group of animals injected on the same day, the 18-hour peak mean of any 20 unselected frogs varied within 10% of the mean of the entire group and the mean of any 10 unselected frogs varied within 25% of the mean of the entire group performed on that particular day. The mean of any 20 unselected frogs taken from experiments performed on different days varied within 40% of the 18-hour peak mean of the entire group. It is therefore obvious that groups of 10 frogs injected on the same day will yield more consistent results than groups of 20 frogs injected on different days. Groups of 20 frogs per dose were subsequently found impractical if a complete assay were to be performed on the same day and hence 10-frog groups were adopted. Any mean obtained from 10 frogs may thus be expected to lie within 25% of the true 18-hour peak mean for the O.W.R. dose on that particular day.

Factors Responsible for Variation

Boyd and Brown (5) investigated a number of possible factors affecting the reaction under study and all of the positive influences established by Boyd and Brown (5) were taken into consideration and standardized in the beginning of the present work. Thus the frogs were kept at a constant temperature of 4° C. in a watery medium of uniform pH and salt content (Lake Ontario water which may be considered more constant in these respects than singly distilled and stored water) and all experiments were performed in the same room.

Air in motion. Boyd and Smith (8) found that imperceptible air currents affected the reaction at room temperature. This was not the case at 4° C. At temperatures below 21° C. the respiratory exchange of frogs may occur entirely through the skin without the added mechanism of the lungs [Dolk and Postma (14), confirmed by Adolph (15)]. At 4° C. it was observed that most of the frogs rested on the bottom of the beakers completely submerged in water with occasionally a portion of the snout exposed. Very few frogs floated or raised themselves above the surface of the water so as to expose a large part of the head, shoulders and back to air, as they do at room temperature. There was thus very little and usually no skin surface exposed to air so that covering the beakers with glass or board to prevent air circulation had little effect at 4° C. while such a technic increased the uptake of water at room temperature (8).

Variation in frogs. All the leopard frogs used in this work were obtained from the same dealer in the Province of Quebec. It has been uniformly noted in this laboratory that the first time a new batch of frogs is used, even after several days in storage, the reaction is substantially more marked than in subsequent injections of pituitrin. At first the possibility of the development of immunity or antihormones was considered but investigation showed that the response of frogs remains about the same after the first two or three injections. If the animals are used in the refrigerator more often than about twice a week, the response begins to fall off. Presumably this is due to some type of cellular fatigue.

Small frogs (12 to 15 gm.) responded as well at 4° C. as large frogs (25 to 35 gm.). In the hibernating seasons of the year, the response declines to a minimum of one-half to one-third of that in midsummer, a seasonal variation which is being studied in more detail (16), but this did not affect the relative response to different doses of

pituitrin. An assay performed in midwinter revealed the same O.W.R. dose as one performed in midsummer.

Non-reacting frogs. In almost any assay at least one frog failed to respond to pituitrin. When several such non-reactors were present in a group, the mean of the group could be considerably influenced if results with all animals were averaged. The non-reactiveness of such frogs appeared to be of a temporary nature because when a group of them was singled out and subsequently injected again with pituitrin, the majority responded normally with an uptake of water.

Red Leg. The only disease which was found to affect these frogs was that commonly referred to as red leg. This assumes epidemic proportions in the summer months but is almost entirely absent in the winter when the frogs are stored in ice-cold running water. The disease was not confined to frogs in tanks, being frequently noted in frogs captured in their natural environment. It is manifested by first an inactivity and flaccidity, followed by a peripheral vasodilation (the 'red leg') and often hemorrhage from the mouth and bowel. Storage of frogs in a cold place and strict sanitary precautions were the most effective methods of control but at most were far from satisfactory. Until within a few days of death, frogs with red leg gave a normal response to pituitrin. A few days later when pituitrin was injected the animals would take up water but instead of losing it they would continue to take up more and more until finally they died within 24 to 48 hours, completely water-logged.

Number of weighings. When the results of the 2 weighing assays at 18-hour intervals were compared with previous experiments, it appeared that repeated weighings at 3 to 4-hour intervals had depressed the response. Investigating this possibility, it was proven that frogs took up more water if weighed only twice, at the beginning and end of 18 hours, than if weighed every 3 to 4 hours. This was considered as further evidence favoring the 2-weighing assay.

The Method of Assay

The method of assay finally worked out may now be described as follows. Fifty frogs were taken from the storage tanks and placed individually in 100 to 150 cc. of water in 400 cc. beakers. The beakers were covered with weighted wire or board tops and arranged in groups of 10 in a refrigerator room at 4° C. and left there for at least 12 hours or overnight. The skin of each frog was then dried, urine expressed and the animal weighed to the nearest 0.1 gm. on a trip scale balance. The pituitary extract to be assayed was then injected with a tuberculin syringe into the dorsal lymph sac and the site of injection thoroughly massaged. Group 3, or the middle group of 10 frogs, was given what was estimated to be about the O.W.R. dose (from preliminary assays with 1 or 2 frogs to a dose; this orienting assay may be done at room temperature and the frogs weighed every hour for 3 to 4 hours or until the peak uptake has passed). Groups 1 and 2 were given lower doses and Groups 4 and 5 higher doses, the total volume of the injection being made the same in each case by quantitatively diluting with saline when necessary. A control group of 10 to 20 frogs should be added at the beginning and injected with saline unless changes in the controls have been previously worked out. The injections are conveniently made late in the afternoon and next morning, 18 hours later, the frogs are again dried and weighed in the same order as on the previous afternoon. The frogs are then brought, in water, to room temperature for 2 to 3 hours, placed in the storage tanks and not used again for several days and preferably not for a week.

The mean weight of each group of frogs before and 18 hours after injection is then determined and the percentage increase in weight of each group calculated. If

a proper range of doses has not been selected, with 2 doses above and 2 doses below the O.W.R. dose, it is necessary to repeat the assay until such a curve is available.

When satisfactory data have been obtained, it is possible to determine the volume of the unknown extract which contains one frog unit of pituitary water retention principle. This unit may be now more specifically and more flexibly defined as follows. A frog unit of pituitary water retention principle is the smallest amount of this principle, per 10 gm. body weight of leopard frogs in water equilibrium at 4° C., required to produce in 18 hours in 10 frogs a mean percentage gain in weight which is within 25% of the greatest percentage gain in weight of at least 3 maximal, plateau means. The figure of 25% is semi-arbitrary being based upon data referring to variation and discussed above.

Example: Oct. 26/38. Assay of Surgical Pituitrin, Batch 728, 257.

Group	Cu. mm. extract per 10 gm.	Mean % gain in weight
1	0.5	7.5
2	2.5	13.8
3	5.0	16.5
4	10.0	21.2
5	25.0	19.6

The highest percentage gain in weight is 21.2%. Twenty-five per cent of this is 5.3% and thus any value above 21.2 − 5.3 or 15.9% increase in weight may be considered a maximal, plateau mean. There are 3 such plateau means and so the results are satisfactory. The smallest dose producing at least 15.9% increase in weight was 5 cu. mm. of this batch of pituitrin and this amount therefore contains 1 frog unit of pituitary water retention hormone or the sample of pituitrin contained 200 frog units of pituitary water retention principle per cc.

SUMMARY

A method is described for the assay of pituitary water retention principle based upon changes in the body water of leopard frogs immersed in water at 4° C. and read in terms of a proposed frog unit.

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THE EFFECT OF THE CRUSTACEAN EYE-STALK HORMONE UPON WATER METABOLISM AND MELANOPHORE EXPANSION IN THE FROG

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IT HAS BEEN SHOWN by Perkins (1) that color in the marine Crustacea is controlled by a hormone elaborated in the stalked eyes of these animals. Smith (2) showed this to hold true also in a fresh water form. Perkins and Kropp have later shown (3, 4) that this hormone is capable of producing melanophore expansion comparable to the effect of pitressin in fish and tadpoles.

In view of the fact that pitressin, commonly used as an activator for amphibian melanophores, has an effect upon the water uptake of these animals (5) it was considered desirable to determine if the eye-stalk hormone (ESH) has the same property. Melanophores were measured at the same time to see if the proportion of water uptake to melanophore expansion was similar to that produced by pitressin. To test this, eye-stalks of the fiddler crab, *Uca pugilator*, were excised and desiccated after the animals had been light adapted for 3 hours. Eye-stalks of the crayfish, *Cambarus blandingii acutus*, were also treated in the same manner.

These forms were used in this work because it was desired to compare the action of the ESH in animals whose water intake problems might differ due to the tonicity of their environment.

The material was injected into 32 frogs following maceration in normal saline solution, the extract being made up as needed. Injections were usually made into the dorsal lymph sac. Following the injection, the frogs were placed in cages containing one inch of fresh water and removed only for weighing and melanophore measurement. Melanophores were measured in the web of the foot by means of the camera lucida, each measurement being the average of 25 melanophores. The measurements are the size of the image of the melanophore when projected by the camera lucida at a magnification of 160. The results of both the melanophore readings and the weight measurements are shown in figure 1.

It may be seen from the figure that the effects on the weight change and melanophore change are similar for the different substances used. The effects were made comparable by using 6 eye-stalks and ½ cc. of pitressin, these injections producing roughly equivalent changes in both weight and color. The *Uca* eye-stalks were found to be slightly more effective in producing melanophore expansion and water uptake although a reverse condition might have been expected in view of the isotonic habitat of the fiddler crab.

In 1934 Abramowitz (6) reported that color changes took place in hypophysectomized animals injected with the ESH as well as in those injected with pitressin although he published no quantitative material. Therefore it was thought desirable to check the relative strength of the pitressin and ESH as determined on the normal frog by injection of these substances into the completely hypophysectomized animal.

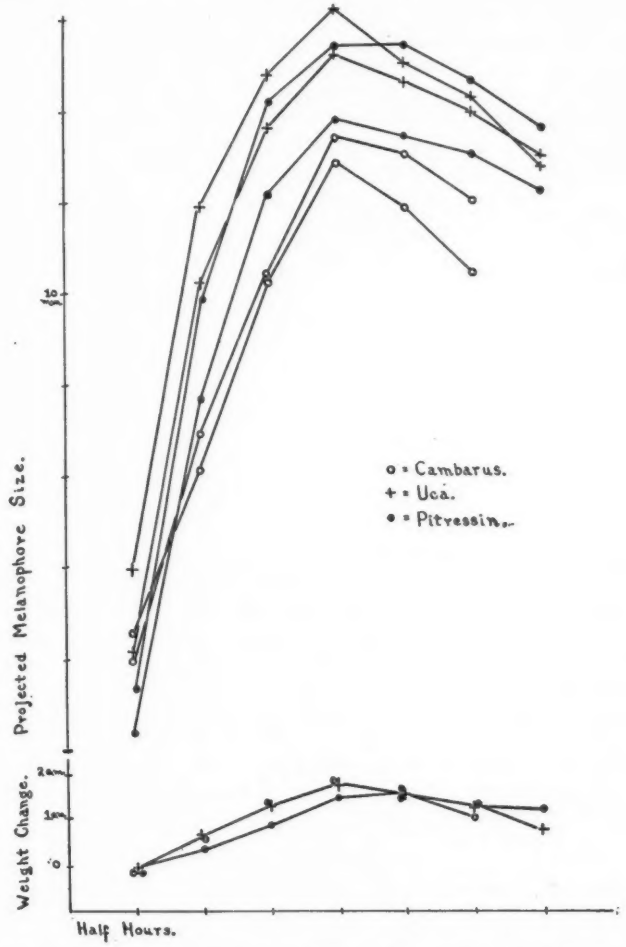

Fig. 1. Changes in melanophore size and weight of normal frogs following injections of eye-stalk extract of *Cambarus*, *Uca*, and of *Pitressin*.

Typical figures are shown in table 1. This confirms the statement of Abramowitz that the ESH does not act by means of the pituitary body but acts directly upon the melanophores.

Table 1. Effects of ESH and pitressin on melanophore size in hypophysectomized animals

	Before injection	Injection	30 min. later	60	90	120	150
Frog 1	4.8	6 ES (*Uca*)	10.1	13.3	13.7	13.2	12.9
Frog 2	5.3	6 ES (*Uca*)	10.4	13.7	13.9	13.5	13.0
Frog 3	5.1	½ cc. pitressin	10.2	13.6	13.8	13.5	12.9
Frog 4	4.7	1 cc. saline (control)	4.9	5.0	4.8	4.6	4.7

The water metabolism effect of ESH having been established, an attempt was made to compare the results with the effect produced by vertebrate pituitary bodies. To this end, the water uptake changes were measured after the implantation of pituitaries of frog, fish (*Ictalurus*), turtle (*Graptemys*) and the mammalian extract, pitressin. The results may be seen in table 2. Roughly equivalent injections determined by this assay seem to be: 1 cc. pitressin = 12 crustacean eye-stalks = 1 vertebrate pituitary. All of these procedures produced melanophore changes as well.

TABLE 2. AVERAGE WEIGHT CHANGES FOLLOWING INJECTION OF VARIOUS SUBSTANCES

No. of frogs used	Injection	90 Minutes after injection	
		Gain in weight	Gain
		gm.	
27	*Uca* ESH	2.4	10.8%
7	*Cambarus* ESH	1.8	7.9%
25	Pitressin, 1 cc.	5.9	19.5%
2	Turtle pituitary	3.6	20.6%
4	Frog pituitary	3.8	17.3%
3	Fish pituitary	5.8	17.5%
20	Control—1 cc. Saline	0.2	0.0%

RESULTS

From the above experiments the following points are clear. It has been confirmed that the crustacean eye-stalk hormone is present in fresh water as well as marine forms. The ESH from both marine and fresh water crustacea has an effect similar to that of pitressin and vertebrate pituitaries upon frog melanophores. The ESH has an effect upon frog water uptake similar to that of pitressin and vertebrate pituitaries. The proportionate effects of the ESH upon water uptake and melanophore expansion are also similar to those produced by pitressin and vertebrate pituitaries.

This similarity implies that unless the melanophore expansion and the water uptake of the frog are effects with a common cause, it is necessary to assume that two separate principles exist in the same proportions to one another in both the vertebrate pituitary and crustacean eye-stalk.

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CLINICAL AND LABORATORY NOTES

THE TREATMENT OF PITUITARY DWARFISM WITH GROWTH HORMONE*

JOSEPH M. LOONEY†

THE PATIENT M.K., a girl of 13, was referred to the Worcester Child Guidance Clinic by the family physician because of retardation in school, timidity, day dreaming, chronic constipation, retardation of physical development and persistent nocturnal enuresis. At the Clinic she was found to have a mental age of 10 years 6 months and an I.Q. of 80. From a psychiatric standpoint there was marked inhibition of speed and psychomotor activity and an active phantasy life. Because of her marked retardation of physical development, she was referred for endocrinological study and treatment.

The child was found to weigh 28.8 kg. and measure 138.7 cm. in height. The minimum normal values for her age would be 35.5 kg. and 146.5 cm.

Past history. She was a full-term baby with normal delivery and weighed 8 lb. at birth. A few days after birth she was jaundiced but the discoloration soon disappeared without treatment. She was somewhat slow in developing because of feeding troubles. She nursed for 6 months and was then placed on the bottle. At the end of the first year she was somewhat underweight. She walked and talked at about 2 years of age and nothing abnormal was noted about her teething. She had no convulsions, fits or seizures but was quiet and placid about the house. When she was 4 years of age her father felt that she was not as tall as others of her age. She had measles and whooping cough at 5 but no diphtheria, mumps or scarlet fever. At 6 it was definitely noted that she was undersized and she was given pituitary powders by mouth, by a local physician, without effect. She has always been constipated and has been given cathartics constantly since infancy. She has had difficulty in controlling her bladder and still has frequent nocturnal enuresis, 3 or 4 times a week. She is very sensitive about this although the other siblings do not tease her. She has done poorly in school and has had to repeat 3 half-year terms. She was in the 5th grade when first seen. No growth had occurred in the past 2 years, according to her father, inasmuch as she still wore the same size clothes.

On physical examination she was found to stand with a marked lordosis and dropping of the shoulders which accentuated her lack of growth. The findings, otherwise, were essentially normal except for a somewhat low blood pressure. Her sexual development was that of a girl of 10 (fig. 1, 2).

The laboratory findings are given in the accompanying table. The urine nitrogen partition showed that the protein intake was lower than normal for a child of her weight, but the distribution was normal. The blood-chemistry determinations gave high values for cholesterol, chloride and phosphorus and a low level for calcium. The differential blood smear indicated a slight eosinophilia and lymphocytosis but the red and white counts were normal. The basal metabolic rate was normal. There was no disparity in the various body measurements and the x-ray pictures of the bones showed normal development for her age. She lacked 8.0 cm. in height from the minimum normal value for her age, and 6.7 kg. from the minimum normal weight.

A diagnosis of pituitary dwarfism was made and treatment with growth hormone was begun.

* I am indebted to Dr. Frederic Fenger of the Armour Laboratories for the growth hormone used in this case.

† From the Memorial Foundation for Neuro-Endocrine Research, Worcester State Hospital, Worcester, Mass.

She was given ½ gr. of thyroid every other day and cod liver oil, 1 tablespoonful t.i.d., and Brewer's yeast, 1 tablet t.i.d. Intramuscular injections of polyansyn (Armour) containing 40 U of growth factor were given every other day. Rest periods of 30 days were given as indicated on the graph.

After 4 months she showed an increase in height of 2 cm. and in weight of 3 kg. A beginning mammary development had also taken place. She was taken off the polyansyn and placed on growth hormone, 50 U, every other day. In January 1938 this was increased to 100 U and in June to 200 U, or 2 cc. of growth factor (Armour) 3 times weekly. The thyroid was increased to 1 gr. every other day in January 1938 and then to 1 gr. daily in June. The Brewer's

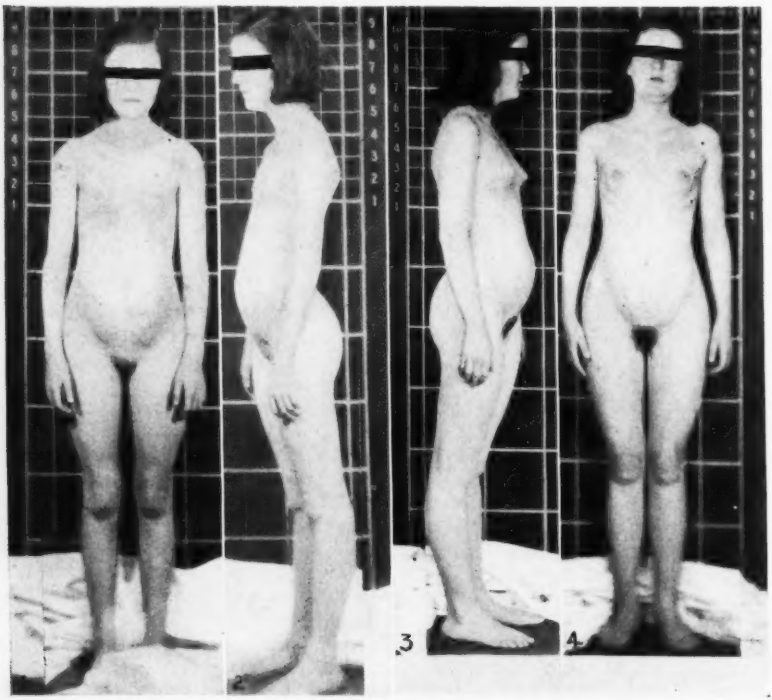


Fig. 1, 2. M. K. Before treatment, May 1, 1937. Fig. 3, 4. After treatment, February 3, 1939.

yeast tablets were increased to 6 tablets daily and she was given 2 gm. dicalcium phosphate daily.

There has been a total gain of 10 cm. in height and 11.8 kg. in weight since treatment was instituted. During this time a normal growth of 6.5 cm. could be expected and an increase in weight of 8 kg., so that even on this basis she has made a net gain of 3.5 cm. in height and 3.8 kg. in weight. She still shows a deficiency in height of 4.3 cm. from the minimum for her age, and in weight of 2.9 kg.

A consideration of the possibilities that could account for this increase in growth leads to 3 alternatives. (*a*). A spontaneous recurrence of growth might have taken place. (*b*). The treatments may have stimulated the child's pituitary to produce sufficient growth hormone to cause an acceleration of growth. (*c*). The growth was a direct response to the medication. Although it is not possible to draw any conclusions from a single case, it seems to me that the evidence favors this last view. It hardly seems possible that a child who has shown

M.K. Child Guidance Clinic

URINES	SP. GR.	REAC.	AMT.	SUG.	ALB.	ACE.	MICROSCOPIC
4-3-37	1012	ac.	820 cc.	neg.	spt.	neg.	Squam. 3-8

NITROGEN PARTITIONS

4-3-37	—	Total Nitrogen	—4.91 gm.	
		Urea Nitrogen	—4.42 gm.—	90.0%
		Uric Ac. Nitrogen—	.07 gm.—	1.4%
		Ammon. Nitrogen—	.13 gm.—	2.6%
		Creat. Nitrogen —	.18 gm.—	3.7%
		Residual Nitrogen—	.11 gm.—	2.3%
4-5-37	—	Total Nitrogen	—6.02 gm.	
		Urea Nitrogen	—5.50 gm.—	91.4%
		Uric Ac. Nitrogen—	.11 gm.—	1.6%
		Ammon. Nitrogen—	.16 gm.—	2.4%
		Creat. Nitrogen —	.23 gm.—	3.4%
		Residual Nitrogen—	.02 gm.—	0.3%

BLOOD CHEMISTRY	CHOL.	N.P.N.	URIC AC.	CREAT.	SUG.	CAL.	PHOS.	CHLOR.
4-7-37	233	26.2	3.46	1.35	101	7.8	4.69	661

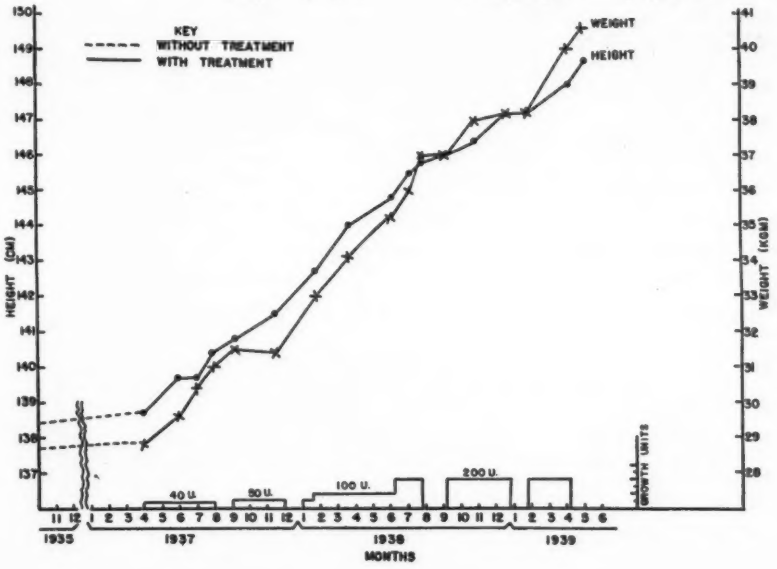
BLOOD COUNT	HB.	R.B.C.	W.B.C.	POL.	LYM.	EOS.	TRAN.	BAS.
4-3-37	15.0 g.	4,940,000	6,900	47.0	45.0	4.0	4.0	0 (200 counted)

X-RAY REPORT

4-3-37 X-ray shows carpal bones and long bones normal for age.
No abnormalities shown in sella turcica which appears within normal limits for size.

BASAL METABOLISMS	Hgt.	Wgt.	Blood Press.	NORMAL BIOMETRIC AVERAGES H.B.	DuB.	Relation of observed to calculated requirement H.B.	DuB.
age—13 years							
4-3-37	138.7 cm.	28.8 kg.	78/38	1091	1241	104	91
						107	94
4-8-37	138.7	29.0	76/40	1093	1241	105	93
						105	93

Measurements				
	Waist	53.0 cm.	Span	134.0 cm.
	Bust	59.5 cm.	Height to symphysis	69.0 cm.
	Hips	71.5 cm.	Sitting height	73.3 cm.



practically no growth would spontaneously show an acceleration more than 50% greater than usually occurs in children of this height. The possibility of stimulation cannot be ruled out but generally it is not found that an organ is stimulated by injection of the hormones which it produces. Furthermore, the fact that a flattening of the growth curve is noted at times when the injections were discontinued lends weight to the view that the gain should be attributed to the action of the growth hormone.

She has not as yet commenced to menstruate but a marked development of the mammary glands has occurred as shown in figure 3.

During this period of treatment the eneuresis has gradually improved so that at present it occurs only at rare intervals. Her school work has greatly improved and she has become jolly and friendly and shows more enthusiasm in her work and play.

THE RESPONSE OF THE OVARIES TO GONADOTROPIC EXTRACTS IN SPLENECTOMIZED RATS

FREDERICK E. EMERY, THADDEUS J. BUGELSKI AND EDWARD L. SCHWABE*

THE REFRACTORY state or antihormone condition induced in animals after prolonged treatment with pituitary extract is apparently not well understood. Selye, et al.[1] induced a refractory state in rats by giving frequent implants of rat pituitaries. Katzman, et al.[2] were not able to confirm these findings in regard to pituitary implants from rats inducing antihormones in recipient rats. Still others, Thompson,[3] Thompson and Cushing[4] and Rowlands,[5] were able to show that the anti-substance is not specific in that antiserum from one animal inactivated pituitary gonadotropic hormone from several species of animals. Gordon[6] has given considerable evidence to show that the spleen is important in the formation of the antihormone. On the other hand pituitary glands from castrated rats implanted into young rats gave large ovaries of approximately equal weight in both splenectomized and in normal rats.[7,8] The present report deals with gonadotropic extracts in splenectomized rats.

Methods. Young rats approximately 30 days of age were splenectomized and started on the extracts. The controls and splenectomized rats were kept in a constant temperature room and fed Purina Dog Chow. All injections were made subcutaneously, usually twice daily, and from the same supply of extract. The experiments were continued for about 30 days, then the rats were killed. The ovaries free from tubes and fat were weighed as was the uterus with the tubes intact.

Effect of antuitrin-S.† In daily doses of 10 R.U. this preparation stimulated considerable growth in the ovaries and uterus. At autopsy the ovaries weighed 114 and 113 mg. and the uterus 258 and 264 mg. respectively in the control and splenectomized groups (Series A, table 1).

Effect of a similar pregnant urine extract. A.P.L. given in 15 R.U. daily induced weights in the ovaries of 61 and 57 mg. and increased the weight of the uterus to 290 and 264 mg. respectively in the control and splenectomized groups. Here again the small mean difference in ovarian weights is without any significance. The fact that the ovaries are smaller in this series than in the rats injected with antuitrin-S is probably due to the difference in assay, the rat unit being larger in the antuitrin-S extract.

Effect of pituitary extract. Since the urine extracts failed to show the spleen was concerned with the amount of ovarian response pituitary extract of sheep was next tried. The results confirmed the urine extracts and showed that the weights of the ovaries and uterus were almost identical in the controls and splenectomized rats (Series C, table 1).

Five of our 88 splenectomized injected rats died before 20 days and are not shown in the table. In 2 of these rats the blood was tested and found negative for Bartonella muris as shown by blood smears (Wright's stain[9,10]) and failure to induce infections when injected

* From the Departments of Physiology and Materia Medica, University of Buffalo, Buffalo, N. Y.

[1] SELYE, H., ET AL.: *Proc. Soc. Exper. Biol. & Med.* 31: 566. 1934.

[2] KATZMAN, P. A., ET AL.: *Endocrinology* 21: 1. 1937.

[3] THOMPSON, K. W.: *Proc. Soc. Exper. Biol. & Med.* 35: 637. 1937.

[4] THOMPSON, K. W., AND H. CUSHING: *Proc. Roy. Soc., London, B,* 121: 501, 1937.

[5] ROWLANDS, I. W.: *Proc. Roy. Soc., London, B,* 121: 517. 1937.

[6] GORDON, A. S.: *Symp. Quant. Biol.* 5: 419. 1937.

[7] EMERY, F. E.: *Proc. Soc. Exper. Biol. & Med.* 37: 455. 1937.

[8] GORDON, A. S., AND H. A. CHARIPPER: *Proc. Soc. Exper. Biol. & Med.* 38: 773. 1938.

† We are indebted to Dr. Oliver Kamm, Parke, Davis & Co. for the antuitrin-S, and to Robert A. Field, Ayerst, McKenna & Harrison for the A.P.L. and gonadotropic extracts, and also to Dr. D. K. Miller of the Department of Medicine for his counsel in regard to Bartonella muris.

[9] FORD, W. W., AND C. P. ELIOT: *J. Exper. Med.* 48: 475. 1928. *Am. J. Hygiene* 10: 635. 1929.

[10] RHOADS, C. P., AND D. K. MILLER: *J. Exper. Med.* 61: 139. 1935.

into splenectomized rats. We have seen Bartonella muris in young rats only once in 70 cases studied after splenectomy performed at various ages up to 80 gm. body weight. Blood smears were taken on the fourth day and at other times up to 30 days after splenectomy. Also the blood (20 tests) when injected failed to produce the infection in splenectomized rats. That young rats may become infected is indicated by Ford and Eliot[9] who showed that it takes about 16 days for the disease to develop after the rat is bitten by an infected louse.

The blood picture in our rats is similar to that reported by others,[11,12] being 8,120,000 and 14.4; and 8,281,000 and 14.8 for the mean erythrocyte count and grams of hemoglobin in the controls (12 rats) and in the splenectomized rats (13 cases) respectively. Both groups were injected with antuitrin-S and killed at 60 days of age. In our older animals (two colonies)

TABLE 1. BODY WEIGHTS IN GRAMS, OVARIAN AND UTERINE WEIGHTS IN MILLIGRAMS OF RATS INJECTED WITH ANTUITRIN-S, A.P.L. AND PITUITARY GONADOTROPIC HORMONES. THE SPLEEN AT THE TIME OF SPLENECTOMY APPEARED NORMAL, THE MEAN WEIGHTS BEING, 166, 157, AND 181 MG. IN SERIES, A, B AND C RESPECTIVELY.

Series	Number of rats	Days injected	Average daily dose	Weights				Mean difference / S.E. of difference	Uterus	Time in estrus
				Body		Ovaries				
				at start	at autopsy	range	mean			
			R.U.							%
A Antuitrin-S	37 Controls	32	10	53.1	134	39–263	114.5	0.2	258.4	34.8
	44 Splenectomized	32	10	52.3	136	32–229	113.4		264.0	33.9
B A.P.L.	23 Controls	30	15	43.8	122	33–143	60.7	0.3	289.6	30.2
	23 Splenectomized	30	15	43.1	115	22–109	57.4		264.2	38.5
C Gonadotropic	16 Controls	28	10	49.0	117	24–198	109.5	1.0	176.0	25.7
	16 Splenectomized	28	10	48.1	116	24–195	92.2		175.4	30.1
D	100 Controls	none	none	—	142	19– 73	46.2	—	—	41.0

Bartonella muris is frequently seen after splenectomy. In 84 splenectomized adult rats 35 died within 12 days and 64 showed positive blood samples.

The extracts used in these experiments were clear and apparently quite free of other substances. A less refined extract might have shown different results. Yet Zondek, et al.[13] contends that even their purest prolan extract was capable of initiating the formation of antiprolan, and that a carrier substance was not the fraction responsible for it.

It is also of interest to note that the ovaries of some rats either do not respond to gonadotropic hormones or have responded and are in a period of retrogression at the time of autopsy. Five of the 44 splenectomized rats injected with antuitrin-S had ovaries below the average of 46 mg. shown for 100 untreated controls. The latter, however, were slightly larger in body weight (series D, table 1). One also finds in the table given by Katzman, et al.[2] that 4 of their

[11] VAUGHAN, S. L., AND F. D. GUNN: *Anat. Rec.* 44: 335. 1930.
[12] LATTA, J. S., AND J. W. HENDERSON: *Folia haemat.* 57: 206. 1937.
[13] ZONDEK, B., ET AL.: *Proc. Soc. Exper. Biol. & Med.* 39: 283. 1938.

10 rats had ovaries weighing 96 mg. or less. This would also be in the range for the ovaries of large normal rats of similar body weight. These observations only add to the confusion of trying to determine if pituitary extract or implants will or will not induce antihormones.

Summary. A total of 83 young rats splenectomized at approximately thirty days of age were injected for a period of 30 days with antuitrin-S, A.P.L. and pituitary gonadotropic extract. The weights of the ovaries and uterus at the end of treatment showed that these organs were not significantly different from those of control rats similarly injected. The ovaries of some of these rats in both the splenectomized and control groups were apparently not increased in weight beyond the mean ovarian weight of normal control rats. The percentage of time in estrus was below normal in all of the injected groups.

CORRELATION OF PITUITARY AND SPLEEN IN EXPERIMENTAL AND CONTROL MICE

J. M. Twort and R. Lyth

Edwards and Wright found that extirpation of the spleen in rats (female) caused an increase in size of the pituitary[1] and we have found that mice bearing experimentally induced malignant skin tumors and rats bearing the Jensen sarcoma, although their spleen may be considerably enlarged, tended rather to show a diminution in size or weight of pituitary compared with tumorless animals.[2,3] We know from previous experience that there is usually a small but definite positive correlation between the weight of the spleen and approximate area or dry weight of the pituitary gland in mice of different total body weights.[4] However we also know that correlation values for total body weight and spleen or pituitary are usually considerably higher than those between spleen and pituitary. The problem we wished to try and solve was whether the size of the spleen per se influenced the size or weight of pituitary.

As we now have at hand data regarding the weights of spleen and relative areas of pituitary in over 4,000 mice and regarding the pituitary dry weights of over 2,500 of these we felt that an analysis of our figures might reveal something of interest in this connection. Since such factors as sex, presence of tumor, malignancy and application of carcinogenic agents could influence the relation between these two organs we divided our mice into 4 groups (*a*) controls, (*b*) experimental tumorless mice, (*c*) experimental benign tumor-bearing mice and (*d*) experimental malignant tumor-bearing mice. Each of the 4 groups were subdivided into males and females. We thus had 8 sub-groups to compare. Nearly all the experimental mice had carcinogenic agents of varying strength applied to the skin over periods of from 25 to 50 weeks usually 5 times per week.

In order to eliminate as far as possible the effect of total body weight we divided the mice comprising each sub-group into weight classes, all mice between 15 and 16 gm. being put into one class and between 16 and 17 gm. into another and so on up to 41 gm. We then found the mean weight of spleen, pituitary and also pituitary area for each class in the different groups. Plus or minus signs were then given to each organ according to whether its value was above or below the mean for its particular class. Thus by comparing the signs against the spleen and pituitary of each individual animal in the different classes and reckoning the number of times the signs were similar or dissimilar we were able to judge as to whether there was any correlation between weight of spleen and area or weight of pituitary in the different classes and groups. Actual mean values were omitted in our calculations except in those mice where both spleen and pituitary gave values equal to the mean for their class. In table 1 is given the percentage number of times the signs were identical for spleen and pituitary area; and spleen and pituitary dry weight in all classes of the various groups. In the table No. is the number of mice in which data as regards spleen, and pituitary area were utilized and No. [1] is the number of these mice in which pituitary dry weights were determined: P.A. is the pituitary area; P.W. is the pituitary weight; C is control; Tms is tumorless; B are mice bearing benign tumors; M are mice bearing malignant tumors. As no obvious differences were noted as regards sex the sub-groups were added.

From the table it appears that there may be a slight positive correlation between spleen and pituitary in mice with the same total body weight. There is, however, no indication of any negative correlation of these organs in any of the groups.

[1] Edwards, L. F., and C. W. Wright: *Endocrinology* 12: 808. 1937.
[2] Twort, J.M., and C. C.Twort: *Am. J. Cancer* 23: 52. 1935.
[3] Twort, J. M., and M. Lasnitzki: *Endocrinology* 23: 87. 1938.
[4] Twort, J. M., and C. C. Twort: *Am. J. Cancer* 29: 78. 1937.

TABLE 1

	No.	Spleen & P.A.	No. [1]	Spleen & P.W.
		%		%
C	579	53	243	61
Tms	2054	52	1406	51
B	870	51	490	52
M	491	50	308	51

Our next procedure was to consider only data relating to those mice in the various classes and groups which had what might be considered abnormally large or abnormally small spleens, the idea being that exaggerated cases might tend to reveal a higher positive degree of correlation or possibly some negative correlation between spleen and pituitary in these particular mice.

Standards of weight can only be arbitrary but here we shall consider all mice in the various classes as possessing large or small spleens when their spleens weighed over 500 or under 100 mg. respectively.

In table 2 are given the results of these analyses in all classes of the various groups. Abbreviations as before and in addition, No. L is the number of large spleens and No. S is the number of small spleens. Under %L and %S are given the % number of times the mice with large or small spleens had pituitary areas over or under respectively the mean values for their respective classes.

TABLE 2

	No. L	% L	No. S	% S
Controls	46	63	11	62
Tms	115	63	50	52
B	57	63	9	22
M	111	56	3	33

As the number of mice with small spleens in the control, benign and malignant groups are so small little value can be placed on these results but it is obvious that when we consider only mice with large spleens we get an increase of correlation of this organ with the pituitary in all groups.

Summary. Data relating to the weight of spleens and approximate superficial area of pituitary gland in over 4,000 mice and data relating to the pituitary dry weight of over 2,500 have been analysed in order to find out whether the weight of the spleen per se influenced the size or weight of the pituitary gland. The mice were grouped according to whether they were untreated controls or experimental and the experimental were further grouped according to whether they bore benign or malignant tumors or were tumorless. The groups were sub-grouped according to sex. Each group was divided into classes according to total body weight (15 to 41 gm.), and the mean value for spleen and pituitary for each class determined. Analysis of our figures revealed that there was a slight positive correlation in all the groups except possibly in the malignant one. No obvious differences as regards sex were noticed in this connection. When mice with large spleens only were considered in the various classes a definite positive correlation was found between the two glands in all groups. There was some evidence of a negative correlation between spleen and pituitary in tumor-bearing mice which had very small spleens but unfortunately the number here were too small to draw definite conclusions. Small spleens of tumorless mice and controls, however, tended to give positive correlation with their pituitaries.

ASSOCIATION NOTICE

ANNOUNCEMENT OF THE ANNUAL MEETING

THE TWENTY-FOURTH ANNUAL MEETING of the Association for the Study of Internal Secretions will be held in New York City on June 10th and 11th, 1940. The Hotel Roosevelt at Madison Avenue and 45th Street will be the headquarters for registration and for the scientific and business sessions.

The Chairman of the Local Committee is Dr. E. T. Engle, 630 West 168th St., New York City.

Instructions regarding the reservation of hotel accomodations will be given all members at the time of the mailing of the annual statements.

PRESENTATION OF PAPERS

Members of the Association and other investigators who wish to present papers will please note the following regulations regarding the program for the coming meetings.

1. The title of the paper, and a comprehensive abstract in *quadruplicate*, must reach the President, Dr. Philip E. Smith, 630 West 168th Street, New York, N. Y., not later than March 1, 1940.

2. Non-members who present papers will appear on the program "by invitation." Non-members are therefore requested to have their titles and abstracts introduced by a member.

3. The abstracts of papers will be considered by the program committee and the final program will be announced about April 15, 1940.

4. Papers will be limited to ten minutes for presentation and five minutes for discussion.

5. All papers presented before the Association or read by title are to be submitted, in completed manuscript form, to ENDOCRINOLOGY, either before or immediately after the meeting. It is to be understood, however, that acceptance of a paper for the program does not obligate the Journal to its acceptance for publication.

You are invited to bring this announcement to the attention of your colleagues and associates who have endocrine research in progrses.

NOMINATIONS OF OFFICERS

The By-Laws of the Association provide that nominations for all elective offices shall be made by a Nominating Committee and forwarded to the Secretary at least sixty days before the annual meeting. The Nominating Committee for the current year, appointed by the President and accepted by the Council, is as follows.

Dr. David P. Barr, *Chairman*, 600 South Kingshighway, St. Louis, Mo.
Dr. E. C. Kendall, Mayo Clinic, Rochester, Minn.
Dr. W. O. Nelson, 1512 St. Antoine St., Detroit, Mich.

The By-Laws also provide (Article V, Section 2) that "Any member of the Association may submit nominations to the Nominating Committee for its consideration." Any nominations which members desire to make should be sent to Dr. David P. Barr by March 1, 1940.

The terms of the following officers expire at the time of the annual meeting in New York in 1940.

President-Elect	E. L. Sevringhaus
Vice-President	Max Goldzieher
Secretary-Treasurer	E. Kost Shelton

Council Members for 3 years	Publication Board for 3 years
J. P. Pratt	Edgar Allen
F. M. Pottenger	M. O. Lee
E. C. Hamblen	

THE E. R. SQUIBB & SONS AWARD

At the Annual Meeting in 1939 the Council directed that the President should appoint a committee to set up the permanent mechanism for making the award. This committee, consisting of Dr. E. L. Sevringhaus, *Chairman*, Dr. F. A. Hartman and Dr. David P. Barr, has drawn up the following regulations for the administration of the E. R. Squibb & Sons Award.

1. In order to encourage investigation in endocrinology, E. R. Squibb & Sons have established an annual award of one thousand dollars to be given through the medium of the Association for the Study of Internal Secretions. The Association through a Committee of Judges selected from its membership, will name the recipient of the award; and the formal presentation will be made part of the annual program.

2. The award will be given to the investigator or investigators in the United States or Canada for one of the best contributions to endocrinology. If in the committee's judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons.

3. A Committee of Judges consisting of five members of the Association will be appointed by the President to recommend to the Council a recipient for the award. The members of this committee shall hold office for five years, one new member being appointed each year. The first year the appointments shall run for one, two, three, four and five years respectively. Nominations for the award may be sent to the Secretary by members of the Associaton not later than January 15 of the year in which the award is to be made.

4. The name of the nominee selected by the Committee of Judges should be sent to the Secretary of the Associaton two months before the annual meeting. This nomination will then be submitted to the Council for ratification and thereafter the nominee will be notified in order that he may make plans for being present at the annual meeting. The name of the nominee will be kept confidential by all concerned until the announcement is made at the annual dinner, at which time the award will be bestowed by the President of the Association. The recipient will be expected to make a brief response.

5. It is desirable that the award be made annually. However, if in any year no suitable candidate is found the Council retains the privilege of witholding the award for that year.

6. In addition to the purse provided by E. R. Squibb & Sons the recipient will be presented with a simple scroll as a memento of the occasion, the cost to be defrayed by the Association.

In accord with paragraph 3 of the above regulations the President has appointed the following Committee of Judges: Dr. E. A. Doisy, for five years; Dr. E. C. Hamblen, for four years; Dr. E. T. Engle, for three years; Dr. F. C. Koch, for two years and Dr. E. Kost Shelton, for one year. Dr. Doisy was named Chairman for the first year. This Committee has made the following announcement.

Announcement of the Committee of Judges

In order that members may have more time to make their nominations for the first E. R. Squibb & Sons Award, the Committee will accept nominations until March 1, 1940.

Each member has the privilege of making *one* nomination. A nomination should be accompanied by a statement of the importance of the nominee's contributions in endocrinology and by a bibliography of the nominee's most important publications in endocrinology. Five copies should be sent to the Secretary, Dr. E. Kost Shelton, 921 Westwood Boulevard, Los Angeles, California, who will transmit a copy to each member of the Committee.

Since the main qualification for the Award is the importance of the nominee's contributions to endocrinology, it seems undesirable for the Committee to make any definite restrictions regarding age of the nominees or the time and place of publication of the contributions.

Philip E. Smith, *President*
E. Kost Shelton, *Secretary*

CURRENT ENDOCRINE LITERATURE

HANS O. HATERIUS, ABSTRACT EDITOR

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ADVANCE ABSTRACTS

Beilly, J. S. Determination of pH of vaginal secretion as an index of ovarian activity in hypo-ovarian states. *To appear in this Journal.*

Further observations with method of determining pH of vaginal secretions in human castrate, menopause and hypo-ovarian groups are detailed. A test, with use of universal indicator, is employed as an index of ovarian activity, enabling one to identify gradient estrogen deficiencies, and, as a guide in the administration of adequate amounts of estrogens. Determination of pH of the vaginal secretions suggested a more flexible classification of vaginal smear types. A revised classification, corresponding more closely to gradient ovarian activity, is offered.

Fellows, M. D. The antigonadotropic hormones in normal human blood serum. *To appear in this Journal.*

A method is described for the determination of antigonadotropic hormones in normal human blood serum, using 19 to 24-day-old female white rats as experimental animals. Normal human blood serum taken during various stages of the menstrual cycle and injected into immature rats failed to produce any inhibition or activation of the growth of the ovaries. The increase in weight of the rat ovaries after injection of 0.6 cc. of a water extract of human anterior pituitary (equivalent to 1.8 mg. dry powder) was 3-fold. However, this growth was not significantly inhibited when 5 cc. of normal human blood serum was given over a period of 5 days (0.5 cc. twice daily) along with the 0.6 cc. of anterior pituitary extract (0.1 cc. twice daily for 3 days). Therefore it is concluded that no antigonadotropic hormone is present in the blood serum of normal human females, detectable by our present methods of assay.

Griffiths, M. The relationship between the secretory cells of the pars nervosa of the hypophysis and classical neuroglia. *To appear in this Journal.*

By the use of the Spanish silver carbonate methods for neuroglia it is shown that Bucy's pituicytes. the secretory cells of the pars nervosa, possess the large ovoid cytoplasmic inclusions (gliosomes) characteristic of neuroglia, and that pituicytes are derived from ectodermal supportive spongioblasts. These spongioblasts form an epithelium about the lumina of the infundibular cavity and its extensions in the pars nervosa. Neuroglial astrocytes and oligodendrocytes are also derived from spongioblasts, but unlike astrocytes, oligodendrocytes are derived from those spongioblasts which never form vascular attachments. Pituicytes and their precursors, however, possess such vascular attachments, so on grounds of genesis and morphology they are closely related to astrocytes. Endocrine functions have been attributed to neuroglia, but the evidence has not been conclusive. The fact that pituicytes have endocrine functions and are closely related morphologically to neuroglia, contributes to the plausibility of the hypothesis.

Herrick, E. H., and C. H. Lockhart. The effect of male sex hormone on the developing ovaries of young fowls. *To appear in this Journal.*

Injections of male sex hormone, testosterone propionate, were made into young, single-comb White Leghorn chicks to determine if these birds had as great ovarian development as their normal controls. Each bird received 0.5 mg. testosterone propionate on alternate days, a total of 5 injections. The chicks were killed at different periods of time after the last injection. The ovaries were

weighed and later sectioned. The total follicles were counted and those 50 micra or over and 75 micra or over in diameter were measured and counted. In 3 of 4 groups the ovaries of injected birds averaged less in weight than their controls. In all groups the injected birds had fewer large follicles than their controls. A group of female fowls 134 days old were given 10 injections of male sex hormone of 0.5 cc. dosage on alternate days (a total of 25 mg. testosterone propionate). The controls of these birds had ovaries 11% heavier than the injected birds. In all injected birds the ovaries averaged lighter in weight or showed less development of the ovarian follicles than their controls.

Hesser, F. H., O. R. Langworthy and S. A. Vest. Muscle Strength in Myotonia Atrophica (Dystrophia Myotonica) Improved by Testosterone Propionate. *To appear in this Journal.*

Two male patients with myotonia atrophica and testicular atrophy, previously receiving only daily quinine, were given 25 mgm. of testosterone proprionate (Perandren) intramuscularly every day for over two months. The dynamometric response of the finger flexors was recorded graphically to note strength (single maximal contraction), total work, and maximal flexion rate before and during treatment. Within the two weeks after treatment strength rose to more than twice the original level and total work was increased by more than half the original amount. This status was maintained throughout the course of therapy and both patients were gratified by the accompanying general constitutional improvement. There were no significant changes in contraction rate. One male patient with progressive muscular dystrophy and normal gonads showed no improvement on similar treatment. Six normal untreated controls showed no appreciable strength changes over an appropriate period. Whether the dynamometric response in the treated cases reflected improved function in the affected muscle fibres or simply a response in the remaining more normal musculature could not be ascertained. Further studies regarding changes in bodily structure, electrolyte and creatine-creatinine balance, and muscle histology are in progress and the role of the adrenal cortex is being investigated. The applicability of androgenic therapy in the treatment of women with myotonia atrophica is under consideration.

Hutton, J. H., J. T. Case, W. L. Culpepper, E. C. Olson and E. E. Madden. The endocrine aspects of essential hypertension and diabetes mellitus. *To appear in this Journal.*

Essential hypertension and diabetes mellitus resemble each other in so many respects as to suggest common etiological factors in many cases. The endocrine components appear to be the pituitary, adrenals and thyroid. Low-dosage irradiation applied to the pituitary and adrenal regions has been followed by symptomatic relief and a considerable reduction in blood pressure in most cases. In some this reduction in blood pressure has been a temporary affair which could not be maintained by subsequent treatments given when indicated by a rise in blood pressure. In many cases the symptoms did not recur when the blood pressure returned to the pretreatment level. A few cases of hypertension have been seen in association with pituitary and thyroid deficiency. In these the blood pressure returned to normal levels and symptoms were relieved following the administration of thyroid and extracts of the anterior and posterior lobes. In a small series of patients having both hypertension and diabetes, the latter appears to have been greatly improved in 50% of the cases, following irradiation of the pituitary and adrenal regions. This form of therapy is an entirely safe procedure and can do no patient any harm if used in the dosages and by the methods we have employed. It seems to possess sufficient promise to warrant careful investigation by other workers.

Ingle, Dwight J. The work capacity of the rat immediately following partial adrenalectomy. *To appear in this Journal.*

The object of the study was to determine the immediate effect of removing varying amounts of adrenal tissue upon the capacity of the rat to continue work of the gastrocnemius muscle. Comparisons were made among animals having sham operations, unilateral adrenalectomy, and a unilateral adrenalectomy with the additional partial ablation of the other adrenal. The removal of one adrenal had the effect of reducing the total amount of work below that of the sham operated animals, and the removal of any additional amount of the remaining gland had the effect of still further reducing the total amount of work performed.—Dwight J. Ingle.

Kurzrok, R., L. Wilson and W. H. Perloff. The action of diethylstilbestrol in gynecological dysfunctions. *To appear in this Journal.*

Forty gynecological cases were studied. The presenting symptoms were menopausal (15 cases), amenorrhea, oligomenorrhea, dysmenorrhea and genital hypoplasia. All were given diethylstilbestrol by mouth or by intramuscular injection. The dosages ranged from 0.05 mg. to 1 mg. once a day by mouth to 5 mg. twice a week by injection. It is concluded that diethylstilbestrol when administered to women possesses the same physiological properties as do the hormonal estrogens. The therapeutic use of diethylstilbestrol is greatly limited because of the persistent nausea and occasional vomiting that is induced in more than half the patients.

LAMPTON, A. K., AND A. J. MILLER. The influence of temperature on the internal secretory activity of transplanted ovaries in the female rat. *To appear in this Journal.*

Sub-adult female rats were castrated, ovaries transplanted subcutaneously into each ear and divided into 2 groups. One group was kept in a low environmental temperature (22 degrees C.), the other in a high environmental temperature (33 degrees C.). At the time the animals were killed (55 to 122 days postoperative) the genital tracts of those animals kept in a low environmental temperature were found to be atrophic, whereas those of animals which were kept in a high environmental temperature were essentially normal.

LANSBURY, J., AND J. HUGHES. Clinical method for estimating monthly output of estrin from single bioassay on pooled night specimens of urine. *To appear in this Journal.*

A method is described whereby the total monthly output of urinary estrin can be calculatd from a single bioassay of an aliquot of the total night urine save for one month. This brings the procedure within the means of the average patient, and permits the patient to engage in normal diurnal activity. The method is based on studies of the relative concentration in the day and night urine and on the comparison of the day and night urine volumes.

McGAVACK, T. H. Masculinizing and non-masculinizing carcinomata of the cortex of the adrenal gland; report of six adult cases. *To appear in this Journal.*

Six cases of carcinoma of the adrenal cortex, confirmed by operation or autopsy, are recorded. Of these, 3 females, age 34, 29 and 38 years respectively, exhibited masculinizing features. The first presented the clinical characteristics and laboratory findings of Cushing's 'basophilism' but at postmortem showed no striking alterations in the pituitary gland. Both of the remaining cases were instances of the adrenogenital syndrome upon which was superimposed a chronic adrenal insufficiency in the later stages of the disease. Of special interest in the third case are the enormous size of the primary mass (1990 gm.), the largest solitary hepatic metastasis recorded, an unusual endobronchial growth, and the absence of a ponceau-fuchsin dye reaction from the tumor or its metastases. Of the non-masculinizing tumors, one occurred in a male age 44 years, the others in females, 70 and 37 years old respectively. Significant in the third was a history of 7 years duration, during the last of which a progressively increasing septic state overshadowed any other features of the picture. The new growth was missed both on physical examination and at operation because of its close adaptation to the substance of the liver. The excretion of estrin, gonadotropic factor of the pituitary, and 'androgen-like substances' has been estimated in the urine of 18 patients, of whom 11 had proven adrenal lesions and four masculinization without demonstrable endocrine involvement. Estrin levels were elevated in cases of carcinoma of the adrenal cortex with virilism. Bitterling tests were positive only in cortical hyperplasias.

PINCUS, G., AND M. GRAUBARD. Estrogen metabolism in cancerous and non-cancerous women. *To appear in this Journal.*

The urines of 5 non-cancerous and 7 women with cancer of the cervix or fundus were separated into estrone and estriol fractions. Urine collections were made before and after the injection of estrone and estrone plus progesterone. In 2 women with normal menstrual cycles injected estrone was apparently converted in part to estriol and this conversion enhanced by simultaneous progesterone administration. In the 3 non-cancerous women without menstrual cycles (ovariectomized, hysterectomized and menopausal) estrone apparently was not converted to estriol. In all 5 non-cancerous women the total estrogen output increased after estrone administration (200 to 500%) and even further after injection of estrone plus progesterone (100 to 150%). In the 7 cancerous women the total urinary estrogen before hormone injection was higher than in the non-cancerous group. After estrone administration the cancerous group exhibited varying increases in total estrogen output (0 to 400%) and practically no further increase with simultaneous progesterone injection. Furthermore the cancerous group's urines gave abnormal estrone: estriol ratios. It is deduced from the data that the cancerous women may convert estrone into a compound different from the ordinary urinary estrogens. A purple pigment occurring in these phenolic fractions shows the solubilities of the putative compound. Data are presented on the occurrence of this pigment in a series of urines from cancerous men and women and in a non-cancerous series. The pigment is found with greater frequency in cancerous persons' urines (about 4 times as often).

ROBINSON, S. K. The age distribution and sex incidence of hyperthyroidism and hypertension. *To appear in this Journal.*

This paper is a study of 157 clinical cases of hypertension and hyperthyroidism, the purpose being to shed further light on the supposed interrelation of these two conditions. Both conditions showed female preponderance of about 3 to 1, with very few cases in the first 3 decades of life. The 5th and 6th decades together accounted for over 50% of all cases. The numerical female preponderance varied greatly with the elevation in both systolic and diastolic blood pressures. As the blood pressure rose, the female preponderance was greater and as the blood pressure fell the female preponderance was less. The systolic and diastolic blood pressures were consistently higher in the

females than in the males, but this was not observed for the B.M.R. There is apparently an antagonism between the B.M.R. and the blood pressure at the very high levels; if the one is very high the other is usually low. Hyperthyroidism and hypertension are closely related conditions. The idea put forward by certain English and German workers that the hyperthyroidism is responsible for the hypertonia, although it has some evidence to substantiate it, cannot be entirely accepted at the present time. It may be true in some cases. A broader view is to look upon the two conditions as resulting from a common underlying neuroglandular disturbance. The blood pressure control center is more resistant to irritation and hypertension is therefore less common in the early stages of the syndrome. When it does make its appearance, it indicates a longer standing disturbance and the prognosis is therefore more serious.

RUBIN, I.C., AND A.M DAVIDS. Pharmacodynamic effects of testosterone propionate on tubal contractions (oviduct of rabbit) determined by CO_2 insufflation. *To appear in this Journal.*

Testosterone propionate was injected into 10 mature rabbits to assay its effects on the tubal contractions. Each animal received 200 mg. of testosterone propionate in divided doses of 25 mg. on alternate days. The tubal contractions were recorded by uterotubal insufflation with CO_2. The results may be briefly stated. (1) Whereas pressures of between 60 to 130 mm. Hg were required to establish patency of the uterotubal junction before injection with testosterone proprionate, patency following injection was established at pressures of between 15 and 30 mm. Hg (2) The tubal contractions oscillated at pressure levels between 40 and 60 mm. Hg before injection, and at levels between 5 and 20 mm. Hg after injection. (3) The amplitude of the individual tubal contractions varied between 5 and 20 mm. Hg before injection, and varied between 2 and 10 mm. Hg after injection. (4) The frequency of the contractions was not significantly altered. The first 3 effects all indicate a definite lowering of tubal tonicity and a marked decrease in the dynamic force of the tubal contractions.

SCHWARZ, H., A. B. NEWMANN AND H. BAUM. Pituitary function in adiposo-genital dystrophy. *To appear in this Journal.*

An investigation of the pituitary function in cases of adiposo-genital dystrophy reveals a tendency (not absolute) toward a greater concentration of 'ketogenic hormone' in the sera of these cases. Children with Frölich's syndrome however revealed the same tendency toward inductions of ketonemia, following fat test meal, the same sensitivity toward the administration of insulin; epinephrin and toward the Staub-Traugott effect. No demonstrable amounts of gonadotropic hormone were found in the sera of the normal children or those with Frölich's syndrome. Genital development was introduced in Frölich's syndrome cases by administration of gonadotropic hormone. There was no effect upon the obesity.

SLEETH, C. K., AND E. J. VAN LIERE. The emptying time of the human stomach after the administration of progestin. *To appear in this Journal.*

The normal emptying time of the stomach was determined fluoroscopically in 4 young healthy adults. The meal consisted essentially of 15 gm. of Quaker Farina. The effect of 1 cc. of progestrin (Upjohn) in oil (one Corner rabbit unit) was then studied on the gastric emptying time. This preparation was given intramuscularly 20 minutes before the standard test meal was eaten. Several determinations, exactly at weekly intervals, were made upon each individual; the average was then taken as expressing the gastric emptying time of that individual. There was considerable individual variation in the response to progestin, but it was found that one Corner unit had no demonstrable effect on gastric emptying in the 4 human subjects.

STEPHENS, D. J. The effect of the thyrotropic principle of the anterior pituitary on the thyroid gland of the undernourished guinea pig. *To appear in this Journal.*

Undernutrition in the guinea pig, of sufficient degree to cause a loss of 20 to 30% of body weight in a period of 2 weeks, results in marked structural changes in the thyroid gland, characterized by atrophy and flattening of the acinar epithelium and retention of colloid, suggesting a resting, inactive gland. These changes are not affected by the administration of vitamin supplements or by the administration of potassium iodide. Refeeding results in a rapid gain in weight and return to thyroid structure to normal. The thyroid gland of the undernourished guinea pig shows a marked increase in sensitivity to the thyrotropic principle of the anterior hypophysis. In such animals structural changes indicate that the thyroid may be stimulated by the injection of as little as one-tenth the minimal effective dose for normal animals. It is suggested that the use of the undernourished guinea pig may increase the sensitivity of present methods for the detection and estimation of small amounts of the thyrotropic hypophyseal principle. Evidence is presented which suggests that suppression of anterior pituitary function occurs in inanition. It is postulated that the structural changes observed in the thyroid gland in undernourished animals may be due to decreased secretion of the pituitary thyrotropic principle. Support for this hypothesis is to be found in the similarity of thyroid structure of the undernourished animal to that of the hypophysectomized animal, the marked increase in sensitivity of the thyroid in inanition

to pituitary stimulation and in the absence of the stimulating effect of potassium iodide.

STONE, C. P. Precocious copulatory activity induced in male rats by subcutaneous injections of testosterone propionate. *To appear in this Journal.*

By daily injections of .62 mg. of testosterone propionate into male rats on and after the ages of 22 to 26 days, the median age of first copulation was set ahead of that of control males by about 20 days. The earliest age at first copulation by an injected male was 29 days; that of an untreated male, 35 days. Probably still earlier copulation may be induced by beginning the injections and the tests somewhat earlier. The pattern of the copulatory act is like that normally appearing in untreated males.

THORNER, J. E. Alopecia totalis: case report. *To appear in this Journal.*

The case of a 32-year old woman with acquired alopecia totalis of 2 years' duration is presented. In the immediate family there were 3 members afflicted with various forms of endocrinopathies (2 with nodular goiters and one with suggestive acromegaly), as well as one sister who has had a recurrent alopecia totalis since early childhood. The results on this patient with anterior pituitary extract followed 5 months later with progynon were striking, but the question still remains as to whether this was merely a spontaneous recovery, or one due specifically to the hormone preparations employed.

VIDGOFF, B., AND H. VEHRS. Studies on the inhibitory hormone of the testes. IV. Effect on the pituitary, thyroid, and adrenal glands of the adult male rat. *To appear in this Journal.*

The effects of the inhibitory hormone of the testes were further studied in relation to the pituitary, thyroid and adrenal glands. In the animals which received the extract the pituitary gland was decreased in weight, and the anterior lobe showed an increase in size and vacuolization of the basophile cells. The thyroid gland was not significantly changed in weight but showed a more uniform size of the acini, larger acini, paler staining colloid, flat epithelium and increase in connective tissue as compared to the controls. The adrenals showed an increase in weight due to hypertrophy and hyperplasia in the zonae fasciculata and reticularis. The changes found were compared with those seen in adult male rats following the injection of estrone and testosterone.

WHEELON, H. Reverse of 'paradoxical' blood sugar response to ingested glucose. A correlation of laboratory and clinical findings on forty patients. *To appear in this Journal.*

The clinical and laboratory findings of 40 patients, each of which showed a reverse or paradoxical blood sugar response to the ingestion of glucose are presented. The average blood sugar readings were as follows: fasting, 140 mg.; 30 minutes following the ingestion of glucose, 123 mg.; 1 hour, 113 mg.; 2 hours, 109 mg.; 3 hours, 101 mg.; and 4 hours, 90 mg. Blood sugar levels of 70 mg. or less occurred 20 times during the course of the glucose tolerance tests. In no instance was sugar found in the urine, either before, during or after the tests. Ninety-nine physical and 248 chemical determinations were made on the 40 patients in addition to those incident to the glucose tolerance tests, urine examinations and 27 Wassermann tests. A total of 39 (40%) of the 99 physical determinations (weight and blood cell counts) lay beyond the limits of normal variation. A total of 107 (43%) of 248 chemical determinations lay outside the range of figures assumed to represent normal variation. Expressed in terms of percentage variation, the deviations were as follows: B.M.R. 58; cholesterol 64; chlorides 43; Ca 43; P 42; urea N 58; creatinine 30. Seventy-two clinical diagnoses, aside from those established by laboratory procedures, were made on the 40 patients. The diagnoses per number of persons affected referable to systems and general states were as follows: nervous, mental and emotional disturbances 73%; diseases referable to the genital organs 23%; cardiovascular-renal diseases 20%; focal and general infections 20%; allergy 18%; avitaminoses 15%; and, skin diseases 13%. A correlation of the findings shows that each of the 40 patients had an average of 5.5 deviations from normal standards in addition to the primary one, namely reverse response to ingested glucose. These findings are discussed in relation to pancreatic and suprarenal functions.

WILKINS, L., W. FLEISCHMANN AND J. E. HOWARD. Macrogenitosomia praecox associated with hyperplasia of the androgenic tissue of the adrenal and death from corticoadrenal insufficiency. *To appear in this Journal.*

A case is reported of a 3½-year-old boy who had precocious development of the male accessory sex organs. A sibling was a female pseudohermaphrodite. The patient had a craving for salt, moderate pigmentation of the skin and gums, and a low blood Na and high N.P.N. He died suddenly. The adrenal cortex was composed largely of androgenic or prenatal zone cells, which apparently replaced other elements of the cortex. The testes were composed of similar cells. To our knowledge, this is the first case in which there is substantial proof that death from adrenal insufficiency resulted from the destruction of the electrolyte-controlling elements of the adrenals by hyperplasia of the androgenic zone. It supports the theory that the androgenic zone has a function different from the portions of the cortex which control the electrolyte balance. Macrogenitosomia

praecox has been reported in the male with adrenal tumors. In the case presented it was due to an embryonic hyperplasia of the androgenic zone, and is therefore analogous to the embryonic virilization which causes pseudohermaphroditism in the female. The familial incidence of such types of sexual abnormality is of interest, and has been reported previously in the literature.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

BREWER, G., P. S. LARSON AND A. R. SCHROEDER. On the effect of epinephrine on blood potassium. *Am. J. Physiol.* 126: 708. 1939.

The transient increase in potassium evoked by epinephrine injection is considerably less in venous than in arterial blood. Infusion of epinephrine in the dog results in the blood potassium attaining a peak level within 2 minutes which is succeeded 1 to 2 minutes later by a comparable fall which may be as great as 35% 10 minutes after beginning infusion. Blood potassium concentration in man following epinephrine injection undergoes alterations identical in kind with those observed in the experimental animal.—From authors' summary.

BRITTON, S. W., AND R. F. KLINE. Augmentation of activity in the sloth by adrenal extract, emotion and other conditions. *Am. J. Physiol.* 127: 127. 1939.

Adrenal extract and adrenalin increased bodily activity in the sloth. Maximum effects of the former was reached in 2 to 4 hours, of the latter in 20 to 40 minutes. The effect of the extract lasted as long as 12 hours.—F. A. H.

DARROW, C. W., AND E. GELLHORN. The effects of adrenalin on the reflex excitability of the autonomic nervous system. *Am. J. Physiol.* 127: 243. 1939.

The injection of adrenalin or the liberation of adrenalin from the adrenal glands leads to a diminished reflex excitability of the sympathetic nervous system. The blood pressure response to stimuli applied to afferent nerves is diminished or disappears completely, the contraction of the normally innervated nictitating membrane is lessened and the difference between reflex pupillary dilatation on the normal and sympathectomized sides following afferent stimuli is diminished or reversed. The galvanic (sweating) response may likewise be decreased. In the case of the parasympathetically innervated sympathectomized pupil the inhibitory effects of reflex stimulation are increased. The experiments thus indicate an inhibitory action of adrenalin on mechanisms controlled by both branches of the autonomic system. The effect on reflex excitability is opposite to that of metrazol and the two pharmacologic agents may counteract one another in this respect. The significance of these observations is discussed from the point of view of 'homeostasis' and 'emergency function.'—Authors' summary.

FITZGERALD, O. The bisulfite binding capacity of blood from normal and adrenalectemized rats and its relation to glycogen metabolism (Die Bisulfit-Bildungsfähigkeit des Blutes von normalen und nebennierenlosen Ratten und ihr Verhältnis zum Glykogenstoffwechsel). *Arch. f. d. ges. Physiol.* 241: 741. 1939.

The bisulfite binding power of the blood is increased by hunger and by adrenalectomy in rats and is inversely proportional to the glycogen content of the liver. This bisulfite binding is in part due to the presence of substances other than acetoacetic acid or other ketone bodies.—A. G.

GROLLMAN, A. The relation of the adrenal cortex to carbohydrate metabolism. *Am. J. Physiol.* 122: 460. 1938.

The purpose of the study was to determine whether the effect of the adrenal cortex on carbohydrate metabolism was primary or secondary. Adrenal cortical extracts, potent as regards their life maintaining capacity in adrenalectomized animals, do not induce hyperglycemic blood levels in normal animals. Amounts of extract employed were 7 to 50 R.U. in the rat and 300 R.U. in the dog. The vital hormone of the adrenal cortex, when administered to adrenalectomized or adrenalectomized-depancreatized animals maintained carbohydrate levels at normal. Purified potent extracts of the cortex did not stimulate ketosis in the normal fasted rat. The adrenal cortex was not responsible for the disturbances in carbohydrate metabolism observed after hypophysectomy since the administration of cortical extract did not relieve them. The above facts indicated that the adrenal cortex was not pre-eminently involved in the maintenance of normal carbohydrate metabolism.—K. A. Brownell.

INGLE, D. J., G. M. HIGGINS AND E. C. KENDALL. Atrophy of the adrenal cortex in the rat produced by administration of large amounts of cortin. *Anat. Rec.* 71: 363. 1938.

Atrophy of the adrenal gland was induced in normal rats by administration of large amounts of cortin. Atrophy of the glands became more marked as the amounts of cortin were increased. Administration of the crystalline compounds A or B produced, in 1 week, degrees of atrophy of the adrenal gland essentially similar to those attained when large amounts of cortin were given. This result definitely shows that atrophy of the adrenal gland is produced by the same crystalline compound

which will maintain the adrenalectomized rat in a normal condition. Atrophy of the gland was restricted to the cortex. The cells were much smaller than those of the normal gland. Cytoplasmic bodies were reduced in size and there were no lipoid deposits in the cells. The extent of atrophy of the glands of animals treated with cortin resembled that which occurs in totally hypophysectomized animals. When an adrenotropic fraction of the anterior pituitary gland was given simultaneously with the cortin, atrophy of the adrenal gland did not occur.—Authors' abstract, Wistar Bibliographic Service.

MacKay, E. M., and A. N. Wick. Influence of adrenalectomy on the blood and urine ketones during fasting and anterior pituitary extract administration. *Am. J. Physiol.* 126: 753. 1939.

Adrenalectomy leads to a rise in the renal threshold for the ketone bodies in the rat so that the urine may be almost or entirely free of these compounds while the blood ketone levels are very high. Assuming a reduction in the blood ketone level to be due to less ketogenesis and not increased ketone body oxidation the blood ketone levels after adrenalectomy indicate only a slight lowering of ketone production during fasting after the removal of the glands. Studies of the blood ketone levels as well as the urine excretion show little effect of adrenalectomy upon the ketogenic action of anterior pituitary extracts. The difficulties involved in the use of the urine excretion rates or blood ketone levels as measures of ketolysis or ketogenic activity are pointed out.—Authors' summary.

Martin, S. J., H. C. Herrlich and J. F. Fazekas. Relation between electrolyte imbalance and excretion of an anti-diuretic substance in adrenalectomized cats. *Am. J. Physiol.* 127: 51. 1939.

Adrenalectomized cats maintained on adequate doses of adrenal extract do not excrete an anti-diuretic substance. However, such a substance is found in the urine 24 hours after cessation of extract therapy. Its appearance is largely due to decreased fluid intake, and not to changes in serum sodium and potassium. After a definite electrolyte imbalance is noted a much larger quantity of the anti-diuretic substance is excreted. Deceased serum sodium from conditions not due to hypoadrenia also results in excretion of a large amount of this substance. The functions of the adrenal cortex and the posterior pituitary are believed to be related in so far as both are concerned with water metabolism.—F. A. H.

Rose, B. The effect of cortin and desoxycorticosterone acetate on the ability of the adrenalectomized rat to inactivate histamine. *Am. J. Physiol.* 127: 780. 1939.

The ability of the adrenalectomized rat maintained on 0.85% saline to inactivate histamine may be restored by the administration of adrenal cortex extract or of desoxycorticosterone acetate. —From the author's summary.

Thorn, G. W., P. Howard, K. Emerson, Jr., and W. M. Firor. Treatment of Addison's disease with pellets of crystalline adrenal cortical hormone (synthetic desoxycorticosterone acetate) implanted subcutaneously. *Bull. Johns Hopkins Hosp.* 64: 339. 1939.

The subcutaneous implantation of pellets of synthetic desoxycorticosterone acetate was followed by striking and continued improvement in 6 patients with Addison's disease. The implantation of pellets resulted, in all of the patients, in changes similar to those previously observed following a daily injection of the hormone in oil, i.e., a positive sodium and chloride balance, an increase in the concentration of these ions in the blood plasma, an increased renal excretion of potassium, an increase in plasma volume, a gain in body weight, an increase in blood pressure and a return to normal activity. In one patient who had an abnormal glucose tolerance curve, continued treatment with pellet implants was associated with a marked change toward normal. Pellets of synthetic hormone are slowly absorbed and thus exert a prolonged effect. The implantation of pellets of synthetic hormone obviates the necessity for daily injections of hormone and, in addition, effects a great economy in the use of hormone. It is desirable to determine first a patient's daily requirement of hormone in oil, injected subcutaneously or intramuscularly, before pellets are implanted. One pellet of 100 to 150 mg. should be implanted for each 0.5 mg. of the hormone required for maintenance.—Author's abstract.

Winter, C. A., W. R. Ingram and E. G. Gross. Effect of pitressin injections upon the serum electrolytes and water exchange of cats with diabetes insipidus and adrenal insufficiency. *Am. J. Physiol.* 127: 64. 1939.

Pitressin administered to adrenalectomized cats with diabetes insipidus prolonged the survival time to that of ordinary adrenalectomized cats and enabled them to develop the usual changes in sodium and chloride which otherwise did not occur. The electrolyte change did not appear to be entirely due to prolonged survival time. Ordinary adrenalectomized cats lost proportionately more sodium than chloride during insufficiency; similarly operated cats treated with pitressin appeared to lose sodium and chloride in approximately equivalent amounts. All the cats showed negative daily water balance following adrenal removal which was of about the same order of magnitude for nonpolyuric cats with and without pitressin, and for diabetes insipidus cats on pitressin, but which was much greater in the untreated polyuric cats. The total water lost during the entire post-adrenalectomy period, however, was slightly less

in the latter group. In no case was the total water loss great enough in itself to account for the death of the animal. Abnormalities in internal fluid shifts are probably more important in causing death than changes in the water balance of the whole organism.

ENDOCRINE GENERAL

FINKELMAN, I., AND A. SIMON. Some effects of the injection of pitressin in dementia praecox. *Illinois M. J.* 76: 287. 1939.

This is a study of 15 cases of acute schizophrenia, 5 patients with manic-depressive psychosis, 4 with epilepsy and 5 normal subjects. Pitressin in doses of 10 U was given intramuscularly. In a number of these subjects the tests were repeated for several days. In the patients with dementia praecox the abnormal motility of the colon, commonly resulting from injections of pitressin, was not observed. —I. B.

HUTTON, J. H. Endocrine disorders from a public health aspect. *Illinois M. J.* 76: 78. 1939.

The problem of recognizing and managing endocrine disorders is one to be solved by the health officer and the private physician. Each has his place. The health officer can probably go no further than to make a tentative diagnosis and suggest to patient or parents that further study be made and the necessary treatment be instituted. From the public health standpoint endocrinopathies may be studied as they occur in 5 different places: 1. school, 2. state institutions, 3. maternal and infant welfare campaigns, 4. industry and 5. the home. In the home the health officer will have very limited opportunities for these observations, except perhaps in cities where the health department has established prenatal and infant welfare clinics. The health officer will rarely have the opportunity and should not be expected to make a positive diagnosis of many endocrine disorders. However, by keeping in mind a few easily recognized signs of these disorders, he can be instrumental in seeing that a positive diagnosis is made and proper treatment be instituted early.—I. B.

LISSAK, K. Liberation of acetylcholine and adrenaline by stimulating isolated nerves. *Am. J. Physiol.* 127: 263. 1939.

Nerves of cats and frogs were stimulated in Ringer's solution containing eserine. The solutions were tested for adrenaline and acetylcholine on the isolated frog heart. The experiments confirmed the previous finding that during activity cholinergic nerve fibers (parasympathetic nerves and ganglia) liberate acetylcholine and adrenergic fibers, adrenaline. Purely adrenergic nerves were obtained by preganglionic denervation of the superior cervical ganglion of the cat and by cutting the vagus below the diaphragm. After degeneration the ganglion and the strands of nerves supplying the gut, when stimulated, liberated no acetylcholine but an adrenaline-like substance. The release of the active substances cannot be regarded as the result of injury because the nerves were not perceptibly deteriorated at the end of the experiment.—E. Bozler.

MACK, C. W., AND B. O. BURCH. Insulin shock therapy in dementia praecox: A report of a series of cases. *California & West. M.* 50: 339. 1939.

The American reports available compare favorably with European statistics on the insulin shock treatment of dementia praecox. A series of 19 cases is reported by the author, with 42.1% recoveries. The relapses in Europe are 6.5 to 23%. The treatment should not be limited to dementia praecox, but deserves wide application in all early functional psychoses.—I. B.

GONADS

ARENAS, N., AND R. SAMMARTINO. Effect of castration and hypophysectomy on the genital tract of bitches (Efectos de la castración y de hipofisectomía, sobre los organos genitales de la perra). *Rev. Soc. Argent. de Biol.* 15: 69. 1939.

Repeated uterine and vaginal biopsies were made before and after operation in 17 animals between 23 and 136 days after castration; in 20 between 11 and 212 days after hypophysectomy and in 6 between 74 and 152 days after hypophysectomy and castration. Castration produced marked regression in animals operated during the follicular and luteal phases, following the normal physiological type of involution; the epithelium retained permanently its anestrus aspect. Hypophysectomy was followed by ripening of a number of follicles; this process ceases promptly and is replaced by cystic follicular atresia; the uterus atrophies at first slowly, and after 3 months, rapidly. In animals with both glands removed the effects are similar to those seen in the other groups.—J. T. L.

ANKER, H. Menstrual disorders treated with follicle hormone (Menstruations-Storungen behandelt mit follikelhormon). *Acta Obstet. et Gynec. Scandinav.* 19: 9. 1939.

Of 7 cases of menorrhagia, 6 of the juvenile type, treated by daily injections of 10,000 M.U. of different preparations of follicle hormone, 2 developed normal menstrual cycles, 3 showed a decrease in hemorrhages followed by amenorrhea for several months and in 2 the hemorrhage was slightly diminished. Of 5 cases of secondary amenorrhea or oligomenorrhea treated with follicle hormone or combined with corpus luteum hormone, 3 reacted favorably. 2 showed enlargement of infantile organs and 1 became pregnant. Hemorrhages came during or immediately following cessation of treatment with follicle hormone. In the second group 10,000 to 50,000 M.U. of follicle hormone were

used. The dose of corpus luteum hormone is not specified.—J. P. P.

Astwood, E. B. An assay method for progesterone based upon the decidual reaction in the rat. *J. Endocrinol.* 1: 49. 1939.

Adult female rats were rendered pseudo-pregnant by electrical stimulation of the cervix uteri during estrus. Four days later the ovaries were removed, and the endometrium traumatized by scratching with a needle point. In 22 control animals whose ovaries remained intact deciduomata developed in every instance. In 16 other uninjected controls whose ovaries were removed at the time of trauma no deciduomata were formed. In the experimental animals progesterone injections were commenced on the day of operation and continued for 3 days. The degree of decidual response was graded by comparing the gross uterine swelling with deciduomata formed in normal pseudopregnant rats. The degree of gross swelling was rated 1 plus to 4 plus. A 4 plus rating was given to these uteri showing a decidual response equal in diameter to those normally found at 3 days. 3 plus equalled normal 2-day deciduomata, 2 plus normal 1-day deciduomata, 1 plus a questionable response. A dose of 1.5 mg. of progesterone was found to give a 3 to 4 plus response; 0.75 mg. a 2 plus response, 0,3 to 0.6 mg., I plus or negative. Estrogen in doses totalling 0.3 μg estradiol simultaneously administered inhibited the reaction. Smaller doses had no effect. Variation in volume of oil or subdivision of the daily dose did not affect the response. It may be concluded that the amount of progesterone which when given over 3 days to animals prepared in this way will result in a 2 plus decidual response is equivalent to 0.75 mg. (0.75 I.U.).—G. B.

Callow, N. H., R. K. Callow, C. W. Emmens and S. W. Stroud. Methods of extracting compounds related to the steroid hormones from human urine. *J. Endocrinol.* 1: 76. 1939.

The paper deals with the effects on the physiological action of the extracts of various procedures used in extracting urine. Studies were made of the effects of variation in temperature, type and concentration of acid used for hydrolysis, time and temperature of boiling, and effects of putrefaction. The same procedures were carried out on samples of both male and female urine. As a result standard extraction methods for urine were suggested.—G. B.

Callow, N. H., R. K. Callow and C. W. Emmons. The effect of administration of testosterone propionate on the urinary excretion of compounds allied to the steroid hormones. *J. Endocrinol.* 1: 99. 1939.

The excretions of 17-ketosteroids and total androgens and estrogens in the urine of male patients treated with testosterone propionate were estimated. The first colorimetrically, the second by intramuscular injection into capons or by inunction on to their combs, and third by injection of phenolic fractions into ovariectomized mice. Three normal and 3 castrated patients were investigated. They received 50 mg. per week and upwards of testosterone propionate. In all cases there was an increase in the urinary excretion of 17-ketosteroids and of androgenic and estrogenic material.—G. B.

De Allende, I. L. C. Effect of estrone on the oviduct of the toad Bufo arenarum (Accion de la estrona sobre el oviducto del sapo *Bufo arenarum*). *Rev. Soc. argent. de Biol.* 15: 185. 1939.

Estrone was injected in doses from 480 to 10,000 I.U. daily during 3 to 10 days. No histological modification was observed. In one-third of the cases injected with the largest dose during 10 days, a secretory activity of the oviduct glands was observed; this effect is not considered physiological. The lack of response to estrone of the oviduct in this species contrasts with what is observed in mammals.—J. T. L.

De Allende, I. L. C. Effect of prolactin on the oviduct of batrachians (Acción de la prolactina sobre el oviducto de los batracios). *Rev. Soc. argent. de Biol.* 15: 190. 1939.

Out of 20 female toads (*Bufo arenarum*) with medium to maximum development of the oviducts 8 showed secretory activity after injecting 1 to 40 mg. The effect was not related to the dose given. Six animals with atrophic oviducts did not respond to prolactin.—J. T. L.

Deanesly, R. Depression of hypophyseal activity by the implantation of tablets of estrone and estradiol. *J. Endocrinol.* 1: 36. 1939.

Estrone in the form of disk-shaped tablets weighing 2.25 to 16 mg. or 80 to 96 mg, and estradiol tablets of the same shape weighing 11.8 to 16.5 mg. were implanted subcutaneously into normal male mice and rats of varying weights. The tablets were implanted aseptically and the skin closed by a stitch under ether anesthesia. The tablets were weighed before and after implantation. The duration of the experiment varied from 10 to 270 days. It was found that this treatment, like the continued injection of estrogens caused inhibition of the functions of the pituitary gland. It led to marked depression of the growth rate, loss of fertility, shrinkage of gonads and accessory organs and enlargement of the adrenals and pituitary. These changes are reversible after removal of the implanted tablets. Three rats with estradiol tablets for 270 days developed tumours of the pituitary gland.—G. B.

Di Paola, G. Cyclical vaginal response to continuous estrone treatment in adrenalectomized castrated rats (Respuesta vaginal cíclica a la es-

trona continua y suprarrenalectomía). *Rev. Soc. argent. de Biol.* 15: 61. 1939.

Daily injection of 1 I.B.U. in castrated rats produced a cyclical response, estrus alternating with diestrus aspect of the vaginal epithelium. Adrenalectomy sensitized the vaginal epithelium to estrone, estrus occurred more rapidly and lasted longer.—J. T. L.

ENGLE, E. T. Current views on the causation of menstruation. *Am. J. Obst. & Gynec.* 38: 600. 1939.

In a review the quantitative relationships of estrogen and progestin effect on the endometrium are presented. The features leading to the estrin privation theory of flow are followed by the difficulties with this point of view. The certainty with which flow follows so soon after withdrawal of progestin effect when there has been preceding estrin action, makes the combined sequence important, although the way in which these 2 hormones lead to bleeding is still uncertain. The changes in endometrial arterioles are presented briefly.—E. L. S.

HAMBLEN, E. C., N. B. POWELL AND W. K. CUYLER. The metabolism and utilization of progesterone given intramuscularly to women. *Am. J. Obst. & Gynec.* 38: 557. 1939.

Studies are reported on 23 women with irregular menses, during 117 cycles of therapy. Endometrial tissue and urinary pregnandiol were the criteria. The authors conclude that injected progesterone was very inefficiently utilized, for they secured very slight evidence of progestational changes at best. The occurrence of pregnandiol was entirely inexplicable.—E. L. S.

HOHLWEG, W. The corpus luteal hormone activity of desoxycorticosterone. (Ueber die Corpus-luteum-Hormon-Wirkung des Desoxycorticosterons). *Zentrlbl. f. Gynäk.* 63: 1143. 1939.

Contrary to the reports of Hoffmann, the progestational activity of desoxycorticosterone is demonstrable only after pretreatment of the infantile or castrate animal with estrogen, and the activity is about one-tenth that of pure progesterone. It is improbable that a conversion of desoxycorticosterone to progesterone is a preliminary to the activity of this type. The tests were carried out on a large series of rabbits.—E. L. S.

LIU, S. H., AND R. L. NOBLE. The effects of extracts of pregnant mare serum and human pregnancy urine on the reproductive system of hypophysectomized male rats. *J. Endocrinol.* 1: 7. 1939.

Different groups of male albino Wistar rats which had been hypophysectomized by a modified Selye technic were injected with varying daily doses of extract of pregnant mare serum and with human pregnancy urine. Hypophysectomy resulted in complete degeneration of seminal vesicles and prostate at 6 weeks. By this time the weights of testicles and adrenals had also reached a steady low level. Immediate treatment with pregnant mare serum (Antex) 0.5 to 1.0 M.U. daily, maintained testis weight and spermatogenesis for the duration of the experiment (42 days). When the treatment was started 2 or 4 weeks after hypophysectomy 1 M.U. daily was not sufficient to bring about much repair, although the rate of degeneration was slowed. When 5 M.U. daily was used, greater restoration of the reproductive organs occurred. Immediate treatment of hypophysectomized rats with extract of human pregnancy urine (Physex) in doses of 0.5 to 1.0 M.U. per day was ineffective in maintaining the reproductive system. The secondary sex organs although somewhat heavier than those of the untreated operated controls remained atrophic. Animals receiving therapy commencing 2 weeks after the operation showed no response with a daily dose of 1.0 M.U., but when 5.0 M.U. daily was used the response of the testes was more marked. The accessory organs were stimulated at first by this dose, but later this effect was lost. When the therapy commenced 4 weeks after hypophysectomy 5.0 M.U. daily produced almost normal testicular weights. The authors consider that pregnant mare serum was more effective as an interstitial cell stimulator but less efficient in restoring spermatogenesis than pregnancy urine extract.—G. B.

LIU, S. H., AND R. L. NOBLE. The effects of extracts of pregnant mare serum and human pregnancy urine on the reproductive system of hypophysectomized female rats. *J. Endocrinol.* 1: 15. 1939.

The ovaries and uteri of hypophysectomized female rats were found to atrophy gradually up to 6 weeks after the operation. After that very little decline of weight occurred. Extracts of both pregnant mare serum (Antex) and pregnancy urine (Physex) were capable of maintaining and restoring ovarian weight but the ovaries were found to regress with continued injections. This regression was much more rapid with Physex. With Antex the uteri remained stimulated and estrus was continuous throughout the experimental period. Mating took place but no pregnancy followed. With Physex a regression in uterine weight followed after initial stimulation. Vaginal estrus was maintained for a short period only. Mating did not occur.—G. B.

MCCAHEY, J. F. A new conception of hermaphroditism. *Surg. Gynec. & Obstet.* 67: 646. 1938.

This is a review of the manifestations in 67 reported cases in the light of Lillie's theory that the primary gonad is testicular in females as well as males. In male pseudohermaphroditism of the external type all the manifestations may be attributed to deficiency of testis hormone activity during the period of development of the wolffian ducts and

urogenital sinus. Female type external genitalia represent the fetal state of the urogenital sinus. The absence of structures derived from the mullerian ducts is significant of genetical maleness. In female pseudohermaphroditism, true hermaphroditism, and male pseudohermaphroditism of the internal form all the manifestations are attributable to a fetal masculinizing action upon the development of genetical females. The cause is failure of regression of the testicular primary gonad which normally occurs in females as the ovary takes form. Fetal masculinization results in complete or partial replacement of ovaries with testis tissue, appearance of wolffian duct derivatives, abnormal terminations of distal ends of female ducts which instead of uniting with the introitus may end blindly in the perineum or connect with the posterior urethra, and partial or complete male type development of the urogenital sinus. Mullerian duct development, although partly arrested in some cases, is constantly found and since this can be attributed to no influence other than that of the ovarian hormone, uteri and Fallopian tubes, signifies genetical maleness. Hermaphroditism in human beings may be divided into 2 forms: that affecting genetical males and that affecting genetical females.—Author's abstract.

McCulleagh, D. R., and W. K. Cuyler. The response of the capon's comb to androsterone. *J. Pharmacol. & Exper. Therap.* 66: 379. 1939.

Androsterone in sesame oil was administered either by injection into the pectoral muscles of Leghorn capons or inuncted by treating the bird's comb for 5 consecutive days. Comb growth was obtained by measuring the increase in length and height on the sixth day. In 15 groups of 14 to 16 birds each, with dosages varying from 10 to 1,000 γ per bird per day intramuscularly, the mean comb growth varied from 1.20 to 18.83 mm. By the inunction method, doses of 0.4 to 20 γ per day in 7 groups of 15 birds each produced a mean comb growth varying from 1 to 14.33 mm. Doses which result in moderate growth, such as 4 to 8 mm., indicate that the inunction method is about 30 times as sensitive as the injection method.—E. L.

Neuhauser, T. The effect of ingested estrone (progynon-DH) and parenterally administered synthetic progestin (proluton) upon the human castrate uterus. *Am. J. Obst. & Gynec.* 38: 609. 1939.

In 6 castrated females the author demonstrated the production three times of progestational endometrium by administering 144,000 active biological units of progynon-DH orally, followed by 55 to 60 U of proluton. In a fourth 90 U of progesterone were used. Two cases failed. Incidentally the estrogen therapy relieved the climacteric symptoms temporarily.—E. L. S.

Newton, W. H., and N. Beck. Placental activity in the mouse in the absence of the pituitary glands. *J. Endocrinol.* 1: 65. 1939.

Fetuses of pregnant mice were destroyed on the 12th day of gestation by bursting the amniotic sacs by external digital pressure through the abdominal wall, while the animal was subjected to light ether anesthesia. The placental tissue remaining after this treatment prevented involution of the mammary glands, and in many cases the cells continued to develop until the alveoli contained secretion. Loss of weight was also prevented and resorption of the symphysis pubis continued. The same results were also obtained in mice which were hypophysectomized by the parapharyngeal method of Thomas (*Endocrinology* 23: 99. 1938). The authors point out that oöphorectomy precipitates abortion and hypophysectomy does so only to a very minor degree, and they suggest therefore a synergic relationship between placentae and ovaries involving a trophic effect of the former on the latter. They suggest also that the presence of placentae in the uterus has a trophic effect on the mammary glands which is independent of ovaries and pituitary.—G. B.

Noble, R. L., I. W. Rowlands, M. H. Warwick and P. C. Williams. Comparative effects of certain gonadotropic extracts on the ovaries of normal and hypophysectomized rats. *J. Endocrinol.* 1: 22. 1939.

Extracts from the pituitary glands of various species, including man, horse, pig, sheep and ox; extracts from the urine and serum of pregnant women, from the urine of a senile and of an ovariectomized woman, and from the serum of pregnant mares were injected into intact and hypophysectomized immature rats. The extracts were injected subcutaneously once daily for 5 days. The animals were killed 24 hours after the last injection. The extracts of the urine and serum of pregnant women and of ox pituitary gland caused luteinization of the ovaries of the hypophysectomized rats; in the intact rats they caused both luteinization and follicular growth. This is thought by the authors to be due to the effect of the luteinization on the pituitary of the intact rat. Extracts of certain human pituitary glands caused follicular growth only in hypophysectomized rats and caused follicular stimulation and luteinization in intact rats. The authors consider this to be due to a normal or to an evoked luteinizing activity by the pituitary gland of the intact rats. Extracts of horse, sheep and pig pituitary gland and of the serum of pregnant mares, stimulates follicular growth and uteinization in the ovaries of both groups of rats. Antisera obtained from rabbits immunized to ox pituitary, injected into hypophysectomized rats which had been treated with human pregnancy urine had no neutralizing action. These antisera had a strong but not complete neutralizing effect

on the same extract of pregnancy urine when injected into intact rats. The authors suggest that this is due to the action of the antisera on the hormones of the rat's own pituitary gland and not on the gonadotropic substance of the human urine.—G. B.

OSTERGAARD, E. Inability of progestin to maintain the decidual phase of the endometrium in rabbits. *Compt. rend. Soc. de biol.* 126: 804. 1937.

In spayed rabbits given estrin and progestin, the uterine changes were observed by means of sections. At the end of 2 weeks, the uterine mucosa showed degeneration after a proliferative stage. Serum from 3 of these animals in the regressive stage was injected into 4 other spayed rabbits whose endometrium was in the transformation stage as the result of injections of estrin followed by progestin. This serum failed to hasten degeneration of the endometrium. In 3 rabbits in which the endometrium had returned to the resting stage after hormone treatment, it responded again with another cycle of activity, after estrin-progestin. The failure of the injections to maintain the endometrium, as in pregnancy, is, therefore, not the result of the development of an antihormone or a permanent resistance to hormone action by the endometrium.—J. C. D.

OSTERGAARD, E. Importance of the synergistic action of estrin and progestin in maintaining the decidual phase of the endometrium in rabbits. *Compt. rend. Soc. de biol.* 126: 807. 1937.

Rabbits were castrated on the 10th day of pseudogestation. The uterine mucosa was maintained without regression for 39 days by daily doses of 10 M.U. of estrin and 3 to 5 R.U. of progestin. With progestin alone, degeneration occurred by the 22nd day.—J. C. D.

SAMMARTINO, R., AND A. ARENAS.—Vaginal biopsy for determination of sex cycle in the dog (Diagnóstico de la función sexual de la perra por la biopsia vaginal). *Rev. Soc. argent. de Biol.* 15: 51. 1939.

Vaginal smears did not give consistent results, so vaginal biopsy was attempted. It was found an easy and innocuous method of following the sexual cycle in this species. The samples were taken by means of a speculum and Hartmann's pincers from the mucosa of the vagina above the urethral opening and not from the vaginal vestibule. During anestrus the mucosa is formed by two layers, the superficial one with mucoid aspect. During the follicular phase marked proliferation takes place, the mucosa has 8 to 10 cell layers, the more superficial are flattened out, but the nucleus remains and no cornification was seen. At the beginning of the luteal phase the mucosa has 15 to 20 layers forming a typical malpighian epihelium, the more superficial form a stratum lucidum, but no cornification was seen. During regression the papillae become flattened and the mucosa is reduced to 2 layers. Pregnant bitches had an anestrous vaginal epithelium, but the mucous was more abundant. Hypophysectomy and castration produced a flat epithelium of 1 or 2 layers of small cells. Injection of estradiol benzoate produced intense proliferation in normal, castrated and hypophysectomized animals. Subsequent progesterone treatment produced a loss of the more superficial eosinophil cell layers. Alkaline anterior pituitary lobe extract produced an estrous reaction in normal and hypophysectomized animals, but not in castrates. Pregnant women's urine and prolan produced a follicular phase followed by a luteal phase in the vaginal epithelium.—J. T. L.

SAMMARTINO, R., AND N. ARENAS. Effect of ovarian hormones on the genital tract of normal castrated and hypophysectomized bitches, (Efectos de las hormonas ováricas sobre los genitales de perras enteras, castradas e hipofisoprivas). *Rev. Soc. argent. de Biol.* 15: 131. 1939.

Estradiol benzoate injected during 5 to 33 days in 20, 200 and 3,000 I.U. daily, produced no effect on the ovaries of infantile and adult bitches. In normal, castrate, hypophysectomized and hypophysectomized castrate bitches, 200 or more I.U. injected during 20 to 25 days produced hypertrophy of the uterus, vagina and vulva and the behavior of heat. The endometrium showed epithelial hyperplasia, with high cells, intense edema of the stroma and a variable development of the crypts; there was considerable hypertrophy of the uterine muscle fibers. The vaginal epithelium was made up of numerous layers, flat and eosinophil on the surface polygonal and basophil in the deeper layers. Progesterone, in doses varying from 5 to 67 I.U., was injected in the course of 5 to 27 days. Previous treatment with estradiol was given in all cases. Out of 12 castrated, hypophysectomized and hypophysectomized-castrated animals only 3 castrates showed a response. The endometrium showed a higher epithelium with abundant and dilated glands, secreting moderately; in the vagina the superficial eosinophil layers were shed. The most marked results were obtained injecting 1, 5000 I.U. estradiol daily for 20 days before beginning the progesterone injections and continuing the estradiol till the end of the experiment. In this species estradiol produced marked proliferation and progesterone added only a slight degree of secretion.—J. T. L.

SHAPIRO, H. Seasonal changes in the ovulation response of Xenopus laevis to methyl testosterone. *J. Endocrinol.* 1: 1. 1939.

Approximately 20 specimens of Xenopus laevis (South African clawed frog) were obtained at each of 4 varying periods extending over 13 months. The animals in each group were injected intraperitoneally with methyl testosterone in doses varying between 25γ and 800γ. Each dose was contained in 2 cc. of nut oil. At least 40 animals were injected at each dose level. It was found

that the dose required to produce ovulation in 50% of the animals was 11-fold greater during the breeding season than at other times. When the response per cent was plotted against the dose injected, a characteristic curve was obtained in each of the 4 experiments. The author considers that the curves which he has obtained provide a basis for a simple and rapid method for quantitatively assaying steroids which are gonadotrophic in Xenopus laevis.—G. B.

SMITH, G. VAN S., AND O. W. SMITH. The anterior pituitary-like hormone in late pregnancy toxemia. *Am. J. Obst. & Gynec.* 38: 618. 1939.

This is a report of 7 years work, with results from 173 pregnancies. Eclamptic toxemias are characterized by serum excess of APL, preceding the clinical signs by 4 to 6 weeks. This fails to appear in about 12%. The serum increase is not present in nephritic or hypertensive toxemias. Premature delivery is sometimes associated with such excess. The findings in the group of diabetics studied follow the same pattern. The authors incline to explain the phenomenon as due to failure to use the APL in the production of increased amounts of the estrogen and progestin steroids, which is a normal characteristic of pregnancy.—E. L. S.

HYPOPHYSIS

BOYD, E. M., E. G. MACK AND A. E. SMITH. Seasonal variation in the response of frog body water to pituitrin. *Am. J. Physiol.* 127: 328. 1939.

After the injection of pituitrin hibernating frogs take up 20% of their weight of water and non-hibernating 10 per cent. Seasonal change did not appear to be dependent on light, temperature, fatigue or dosage. It was suggested that the difference in response may be associated with the sexual cycle.—F. A. H.

CLARK, G., AND S. C. WANG. The liberation of a pressor hormone following stimulation of the hypothalamus. *Am. J. Physiol.* 127: 597. 1939.

In 7 of 10 low cervical cats, stimulation of the anterior hypothalamus yielded pronounced and sustained pressor effects. The long latent period, the slow rise of the blood pressure to maximum and its persistence after vagotomy further exclude the possibility that it is of a nervous origin, or due to circulating sympathin or adrenin. An activation of the hypophysis is therefore presumed and the effect is accounted for by the liberated pressor hormone from the posterior lobe of the pituitary gland.—Authors' summary.

ENNOT, A. H. The influence of anterior pituitary extracts on the content and rate of oxidation of glutathione in tissues. *Aust. J. Exper. Biol. & Med. Sci.* 17: 173. 1939.

It was found that the administration of anterior pituitary extracts to rabbits produced no definite alteration in reduced blood glutathione or in the oxidized form. The extracts were found to produce a marked lowering of the reduced glutathione content of liver particularly with saline extracts. An inhibition in the rate of oxidation of reduced glutathione in liver from treated animals was also demonstrated.—G. B.

FEVOLD, H. L. Chemical differences of the follicle stimulating and luteinizing hormones of the pituitary. *J. Biol. Chem.* 128: 83. 1939.

The follicle stimulating hormone is precipitated and inactivated by picrolonic, picric and flavianic acids. The reaction is reversible and the activity is regenerated when the acids are removed from the precipitates by neutralizing with 10% NaOH and precipitation of the active material by 10 volumes of cold acetone. The luteinizing hormone is not inactivated by these reagents and is as effective by intraperitoneal injection as by subcutaneous injection. The follicle stimulating hormone is only effective when injected subcutaneously, and while incapable of stimulating the interstitial cells of the testes to secrete male hormone by itself, it augments the action of the luteinizing hormone in this respect.—J. M. L.

FREUD, J., L. H. LEVIE AND D. B. KROON. Observations on growth (chrondrotropic) hormone and the localization of its point of attack. *J. Endocrinol.* 1: 56. 1939.

In 70 hypophysectomized rats (45 days old and weighing 80 gm.) there was closure of the epiphyses of the tail vertebrae and a cessation of tail growth. Seven days after the operation the difference between operated and control animals could be readily detected in skiagrams of the tail. Once the epiphyses were united, treatment of the animal with growth hormone (from acetone dried or fresh beef pituitaries) had no effect. If the growth hormone is injected immediately after hypophysectomy, epiphyseal closure is prevented and normal longitudinal growth of the tail is maintained. The authors suggest that the tail length and vertebral development as shown by serial skiagrams can be used for the assay of unknown preparations believed to possess growth hormone activity.—G. B.

HAIR, G. W. The nerve supply of the hypophysis of the cat. *Anat. Rec.* 71: 141. 1938.

The hypophysis of the cat receives unmyelinated nerve fibers from the carotid plexus. These fibers reach the gland by accompanying its arterial channels and are distributed to the pars tuberalis and anterior lobe and, in smaller numbers, to the posterior lobe. The fibers are not entirely of sympathetic origin since cervical sympathectomy does not lead to total degeneration of intraglandular fibers. The pars nervosa and pars intermedia receive an abundance of fibers which take origin in hypothalamic nuclei. A few fibers from the same

source penetrate the infundibular stalk to reach the pars tuberalis, while an occasional one has been traced to the anterior lobe. The nerve fibers ramifying in the epithelial parts of the hypophysis have knob-like enlargements which lie against the epithelial cells of the part. The fibers of hypothalamic origin in the pars nervosa terminate as coiled endings having a definite protoplasmic sheath.—Author's abstract, Wistar Bibliographic Service.

INGRAM, W. R., L. LADD AND J. T. BENBOW. The excretion of antidiuretic substance and its relation to the hypothalamico-hypophyseal system in cats. *Am. J. Physiol.* 127: 544. 1939.

Normal cats subjected to conditions producing dehydration excrete antidiuretic substance in the urine according to bioassay on rats. Cats with diabetes insipidus due to interruption of the supraoptico-hypophyseal tract without direct injury to the hypophysis do not excrete this substance when dehydrated. When commercial post-pituitary preparations are injected in normal cats and cats with diabetes insipidus, antidiuretic substance appears in the urine in quantities which are relatively small as compared with the amount administered. The results indicate that the neurohypophysis may respond to the need for water conservation by secreting increased amounts of antidiuretic hormone, and indicate that the supraoptico-hypophyseal tract is necessary for this response.—From authors' summary.

LOEB, L., AND S. J. HAYWARD. Further investigations concerning the effects of formalin on the gonadotropic and thyroid-stimulating hormones of cattle anterior pituitary glands in the guinea pig. *Am. J. Physiol.* 127: 497. 1939.

Immersion of cattle pituitaries in 0.25% formalin caused reduction or suppression of the luteinizing and atresin effects and in many instances a full maturation of follicles in the ovary of the guinea pig, the mature follicles inducing heat changes. A definite reduction in the luteinizing effects in the ovaries of the guinea pigs was a prerequisite for the production of fully mature follicles and of the typical heat changes in the secondary sex organs. It was possible to preserve these specially treated pituitaries in glycerin for months. Formalin injured or destroyed the thyroid hormone independently of the luteinizing or atresin effects.—From author's summary.

WALKER, A. M. Experiments upon the relation between the pituitary gland and water diuresis. *Am. J. Physiol.* 127: 519. 1939.

An antidiuretic effect was obtained from the blood of rabbits and urine of rats which had been dehydrated. It was concluded that the substance responsible was probably not identical with the antidiuretic fraction of pituitrin. By modifying the experimental conditions it was possible to cause the distinction between hydrated and dehydrated animals to disappear. When present the amount of antidiuretic substance seemed to be insufficient for physiological importance. The antidiuretic substance found in cerebrospinal fluid and in urine was not abolished by removal of the posterior lobe of the pituitary. It was also found in the urine of hydrated or moderately diuretic animals. Hypophysectomized cats and rats developed diuresis or oliguria with water ingestion or deprivation in a fashion quantitatively similar to that which they exhibited before operation.—From author's summary.

PANCREAS

BARACH, J. H. Growth in diabetic children. *Pennsylvania M. J.* 42: 1459. 1939.

The well controlled diabetic can grow, mature, reproduce, and meet all the requirements of life in a normal way. In diabetes involvement of the endocrines is not to be considered as an isolated complication. The pituitary gland influences carbohydrate, protein and fat metabolism; the thyroid gland influences protein metabolism; the parathyroids control the calcium-phosphorus metabolism; the pancreas controls the insulin mechanism; and the adrenals drive the glucose metabolism. Thus the rôle of the endocrines is highly complex and it implies an inextricable physiologic process. Nor is it usually a simple matter to tell whether the mineral supply of an organism alters endocrine functions or whether hormones direct the mineral metabolism. There are facts which favor both views. In either case, they are dependent on one another and they complement each other in the functioning of the organism. The author's observations cover a series of 75 young diabetics. About half were of normal height and growth, while the other half were above or below the average. It is in the latter that he found the definitely under- and overgrowth cases. The author found that hyperglycemia and glycosuria, of themselves, were not the cause of asymmetrical or subnormal growth. The diabetic child or youth needs food for both maintenance and growth. He must be given plenty of calories, supplied by carbohydrate, protein, and fat in physiologic proportions. These foods, properly chosen and including a generous portion of milk daily, will also contain the necessary minerals and vitamins. Insulin is given when needed, aiming constantly at a sugar-normal state of blood and urine. If, regardless of this treatment, the patient shows a lag in growth, then endocrine preparations are in order. The hyperthyroidism may be corrected with desiccated thyroid substance in proper dosage. The pituitary element is dealt with by the use of an extract of the anterior lobe of the pituitary. If the growth element alone is indicated, the pituitary growth hormone may be used, and if there is gonadal deficiency alone, then the pituitary sex hormone may be emphasized in the treatment.—I. B.